The Hemicelluloses of Straw and Wood

by

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INTRODUCTION

The term "hemicellulose" was first attached by Schulze (1) to those cell wall materials which he considered to be in some way related to cellulose, but which were distinct in their ease of hydrolysis to simple sugars. Schulze found that such hemicelluloses could be extracted from wheat bran, leguminous seeds, and other plant materials with dilute alkali and precipitated with alcohol, and he regarded them as hexosans, pentosans, or hexopentosans. As data on the hemicelluloses has amassed during the past sixty years, the simple, original conception and definition have undergone profound modification. It is now recognised that the hemicellulose of any one plant or wood is not necessarily a discrete substance, but may be a mixture of polysaccharides having two possible sources, and probably three broad functions. Part of the hemicellulose is considered to be bound to the true cellulose as cellulosan, whilst the remainder is free from association with the cellulosic fraction. This remainder may be further subdivided into polyuronides, which contain some proportion of uronic acid, and the polyoses, which have none. The cellulosans, interlaced with the cellulosic framework have a doubtful function; the polyuronides are thought to form an encrusting substance together with the lignin, with which they have a problematical association,

whilst the polyoses may be the reserve substances of the plant.

With such a heterogeneity of material present in normal plant tissue, it is not to be expected that by simple alkaline extraction one could differentiate sharply the various polysaccharides present. Much of the early work was done on materials which have subsequently proved to be mixtures. The advent of chromatography has done much to ensure higher standards of purity, yet, even now, should a cellulosan occur together with a polyuronide having the same basic sugar residue, the mixture might not be recognised as such, nor could a separation easily be effected if the presence of two polysaccharides were appreciated. A possibility is that the present subdivision of hemicelluloses will be reorganised. It is only within recent years that the comparative ubiquity of uronic acids has been recognised, and as a result, the number of well authenticated polyoses is now small. The number of known cellulosans is also decreasing. Residues of ∝ cellulose from various sources thought to contain small quantities of associated pentosan on the evidence of furfural yields are particularly open to doubt, unless pentosan has been proved to be present by other means. Sarkar, Mazumdar and Pal (160) found jute ∝ cellulose to be chromatographically free from pentosan, although up to 0.9% furfural may be given on analysis. The occurrence, however, of small amounts of associated hexosan and pentosan in ∝ cellulose from wood, appears

well established.

As a general term of reference, "hemicelluloses" may be said to embrace those cell wall polysaccharides which are extracted from plant tissues with dilute alkali, but not with water, and which may be hydrolysed to their constituent sugars with dilute acids (46).

Hemicelluloses are of wide occurrence, being found in all plants, and marine algae. Amongst the sugars found on hydrolysis, xylose is prominent. In 1889, three years before the term "hemicelluloses" was originated, Wheeler and Tollens (74) obtained xylose from beech wood sawdust, and in the following year, Allen and Tollens (75) found the same sugar in cherry wood. It was soon afterwards isolated from wheat straw, maize stalks (76), maize cobs (77), and cucurbita seed husks (78). The first isolation of xylan, or Holzgummi, is due to Thomsen (79), and Poumarède (80). Thomsen extracted resin free beech sawdust with cold alkali, and precipitated the product with alcohol. He studied the variation of xylan content with the age of the wood, and showed it to be present in the wood of deciduous trees, but failed to find it in coniferous woods. It gave no colouration with iodine, and he considered it to be isomeric with cellulose. Later investigations by Wheeler and Tollens (74,81), and Allen and Tollens (75), in which 'Holzgummi' was isolated from various materials by Thomsen's method, revealed its nature as a pentosan.

Following the early work on hemicelluloses, a number of theories on the mechanism of lignin formation

were advanced. Cross and Bevan (58) suggested that it could be regarded as a metabolic product of cellulose, and this theory found support from a number of early workers (17,57). During later years, however, pentoses, methyl pentoses, and hexoses have all been suggested as possible precursors (50,51,52,53,56). Klason (56,59), Rassow and Zschenderlein (60), and others have suggested that lignin is formed by the plant from pentoses or pentosans, paticularly xylan. Schrauth (50) has put forward a possible dehydration mechanism for this. Buston (61) considered it more likely to have arisen from glucosan-xylan type polysaccharides. Pectin has also been cited as a possible precursor (62), in view of the fact that young, non lignified tissues are rich in pectin, whereas the older plants are deficient in this material (63). As against this, the possible formation of hemicelluloses from pectin has been advanced (61), on the basis of the latter's ready oxidation to products resembling hemicelluloses by Fenton's reagent at 30° in vitro (69). In criticism of these hypotheses it has been pointed out by various workers that whilst the absolute percentage of lignin increases with the age of the plant, it does not do so at the expense of pectin, pentosans, or cellulose. Rather does the absolute quantity of all these materials show a simultaneous increase with age (61,65,66). The position is further complicated by the fact that lignin of different species is not identical, and indeed different types of lignin may be present in the same plant. Philips and Goss (72)

have separated three distinct fractions of lignin from wheat straw.

There is now a considerable weight of evidence in favour of a polyuronide hemicellulose-lignin link in vivo. As examples may be quoted the well known difficulty of dissolving any but a small proportion of hemicellulose present with dilute alkali unless delignification is first effected; the impossibility of methylating lignin in situ, and the higher chlorine uptake of isolated lignin. In early work, a lignin-cellulose combination was suggested (33,34,35), but the later cellulose micelle theory, and other evidence, made this untenable (43). The modern theory of a hemicellulose-lignin link has found support from studies of enzymic degradation of wood. Freudenberg and Ploetz (31) have shown that degraded linden wood fractions which are attacked by enzymes leave a residue of enzyme resistent material having integer lignin-carbohydrate molar fractions. Observed lignin-carbohydrate ratios of 1:1 and 1:3 have been cited. Claims to have isolated a lignin-carbohydrate compound have been made in recent work (32) on yellow birch wood, the carbohydrate portion of the compound being shown to be partially D. xylose. The lignin compound was extracted from the moisture free woodmeal with boiling glacial acetic acid, over a period of four days. Other types of linkage have been postulated by various workers. Xylan-cellulose links have been discussed by Nevros (45), Ludtke (44), and Schmidt (153). Nevros has claimed the isolation from alder of a distinct entity having

residues in the ratio of 3 glucose to 1 xylose. Schmidt (153) has advanced other evidence in accord with this finding.

Whilst the evidence for a hemicellulose-lignin link appears appreciable, the nature of such a link is by no means clear. Suggestions have included an ester link between a hemicellulose hydroxyl and an acid group in the lignin (34,37); an ether link between lignin and hemicellulose (38,39,40); an acetal type link (41), and a mixture of ether and acetal (42).

The variation of hemicellulose type, and content, with the age of the plant and conditions of growth, has also received attention. Early work by Thomsen (79) indicated the xylan content to be greater in new than old wood, and to increase as the axis of the trunk was approached. Spring wood contains more pentosan (xylan) than Summer wood (85,86). Substantial differences between the hemicellulose of oak heart and sapwood have been demonstrated by O'Dwyer (93,95,112). Buston (61) has cited evidence to show that the hemicelluloses of the bean and vine, both after slow starvation and slow drying, differ appreciably from that of the green samples. He has also shown that leaves of various plants starved for up to 2 months before analysis, show a loss of hexosan and pentosan - mainly from the hemicellulose B fraction - and an increase in the uronic anhydride. Moreover, the xylan which is extracted from starved samples appears to be physically different from normal, as manifested by its precipitation as hemicellulose A

by acids from alkaline solution. It has been pointed out by earlier workers (161,162), that slow drying might be expected to favour an accumulation of pentosans and anhydride. O'Dwyer has shown (93) that although the yields of hemicellulose A from oak sap and heart-wood may be reduced as a result of drying the wood under conditions of increasing severity, the relative amounts of uronic acid and xylan are not altered. Meller (163) has recorded that increasing amounts of pentosans resistant to alkaline extraction are obtained whenever wood pulps are dried at high temperatures. However, an investigation into the composition of various cereal straws by Ritman, Muller and Dijkheis (71) indicated that pentosan, holocellulose and lignin contents of Summer and Winter wheats, from light and heavy soils, showed only insignificant differences. In fact, there was suprising correlation between analysis figures for various straws. All sampleas of wheat, oats, and barley straw examined had a pentosah content, based on oven dry material of 26-27% and holocellulose 69-71%. No hexosans were found in the hemicellulose.

It is of interest to note how the xylan content of different hemicelluloses may modify the properties of the parent material. The common fibres such as jute, cotton, hemp, kapok and others, may be divided into two broad groups on the basis of the xylan content of the derived cellulose. The fine fibres, such as flax, and ramie, yield celluloses with small amounts of xylan as cellulosan, whilst the celluloses from the

coarser fibres such as manilla, sisal, and kapok have considerably higher xylan contents (82). Norman (28), reporting on analyses of a wide range of materials, states that the two groups do not overlap. It is possible, therefore, to subdivide all plant tissues so far examined, into low xylan types, possessing 0 to 6% xylan, and high xylan types with 13 to 25% culminating in the hardwoods and straws. No plant tissue yielding an α cellulose with xylan content between 6 and 13%, has so far been authenticated.

One of the main differences between hard and softwoods also lies in the xylan content. In hardwoods, the pentosan content - mainly xylan - rarely drops below 17% and is often in excess of 21% (84), whereas in softwoods the pentosan varies between 8 and 14%. The hemicellulose of hardwoods is higher than that of softwoods, since the 4 - 10% mannan, which the latter contains, does not recompense for the loss in pentosans. Hardwoods contain little (92, 113, 127), or no mannan (114, 125, 126). The quantity and type of lignin present in the two species of woods also differs.

As has been mentioned, extraction of hemicelluloses, such as xylan, is usually achieved using dilute alkali, although other methods have been used. Thomsen (79) used cold, dilute, sodium hydroxide to extract xylan from deresinified, beech sawdust. Following his investigations, some workers have used boiling alkalis (22, 115, 116, 154), but this was later discontinued

as possibly having a degradative effect and as unnecessarily harsh, particularly if extraction of holocellulose rather than untreated tissue is desired. Wise (117) has examined the effect of graded extraction of pine, spruce, oak and other wood holocellulose with potassium hydroxide solution under nitrogen. He used concentrations of 2, 5, 8, 10, 12, 16 and 24% alkali, and found that removal of hemicellulose is rapid up to a concentration of 10%, after which the residue shows only small losses in weight with succeeding increased concentrations of alkali. He showed that if alkaline extractions were carried far enough, air being rigorously excluded, successive extractions no longer caused any change in the amount of fibrous residue. Ordinarily, if α cellulose is retreated with alkali in presence of air it shows a marked loss in weight (118). Unfortunately, figures for pentosan content of Wise's α cellulose were not determined. It would be of interest to know the proportion of xylan, if any, present as cellulosan. Other methods of extracting xylan have varied in severity according to the lignin present, but include alkali with 3% sodium chloride, alkali under pressure (73, 124), and sodium carbonate solution (99). An interesting exception is the xylan from Rhodymenia palmata (68), which was extracted with 1% hydrochloric acid.

After the initial work on crude Holzgummi, efforts were made to secure a purer product both by prior removal of interfering substances, and by purification

of the extract. Thomsen (79) gave beech sawdust pretreatment with ammonia before extraction. Most later workers have removed lipids with organic solvents before hemicellulose extraction. Dilute ammonium oxalate has been used to remove pectic substances (87,119). The nitrogen content of woods and straws is low, so that prior removal of protein is unnecessary. Adams and Castagne (131) have shown that acid chlorite delignification of wheat straw results in almost complete removal of the small quantity of protein originally present in the straw.

Delignification

In early work the hemicellulose was extracted from the lignified tissue, and the dilute alkali used caused solution of some lignin along with the hemicellulose. Its subsequent removal was not easy (93,134). After many reprecipitations a preparation may still contain 3 to 5% of lignin, whilst the content of a crude extract may be as high as 15% (19). Methods were advanced by which lignin could be removed before alkaline extraction. Since straw and wood are both heavily lignified, delignification methods were particularly sought which might be suitable for use with these materials. The methods available, and their effect on the accompanying xylan, will here be briefly considered.

The original method of delignification, due to Cross and Bevan (111), made use of alternate treatments with chlorine gas and boiling sodium sulphate solution, and

removed hemicellulose as well as lignin, to leave a residue of "true" cellulose, and cellulosan. It is thus of little value as a preliminary to hemicellulose investigation, although modifications of the Cross and Bevan method intended to minimise hemicellulose losses have been suggested (2,3). The first method by which isolation of the total carbohydrate fraction in plant tissue could be approximated was that of Schmidt and Graumann (4). Alternate solutions of chlorine dioxide and sodium sulphite were employed, with the latter being later replaced by aqueous pyridine (5), to increase the yield. This method required a month for complete delignification of a typical wood sample. In 1933, Ritter and Kurth (6) suggested the use of successive treatments with gaseous chlorine and alcoholic pyridine After three such treatments over a period of ten hours, followed by removal of the residual lignin with cold dilute calcium hypochlorite solution, they obtained from extracted maplewood a residue containing over 96% of the pentosans originally present in the wood. This residue, the holocellulose, contained all the acetyl groups of the original material, as did Schmidt's "skeletal substance" and, in addition, all the carbon dioxide yielding material, as compared to 87% by the Schmidt method. These results were substantiated by other workers (7). In later work, the procedure was modified by eliminating the final bleach (8), and by the use of a hot, alcoholic solution of ethanolamine in place of pyridine (9). High apparent yields of holocellulose by this method have recently,

however, been shown to be due, in part, to the balancing of the slight loss in hemicellulose by a retention of nitrogen as ethanolamine, or ammonium salts (10,12). This is supported by the high nitrogen content of the hemicellulose, and by the fact that some pentosan may be recovered from the aqueous washings between successive treatments. Thomas (13) concludes, from a series of determinations on aspen wood, that even using a minimum of iced wash water there results an appreciable loss of hemicellulose. Since it is impossible to complete the reaction within a reasonable time under anhydrous conditions (2), this is a consequence which cannot easily be avoided and may well be found to apply to other delignification procedures making use of aqueous washes (14).

A third general method for the isolation of total carbohydrates in plant tissues is due to Jayme (15), and has been modified by Wise (12,13). It is based on the liberation of chlorine dioxide from sodium chlorite and acetic acid. A small amount of tenaciously held lignin is allowed to remain, since attempts to remove this result in the loss of some carbohydrate material (12,14,15,131). Staudinger and Jurisch (18) have claimed that chlorine dioxide has a degrading effect on cellulose and so, presumably, on hemicellulose. The possibility has been supported by other workers on spruce and beech hemicellulose (20,21). Atchison (10) suggests that chlorine water may have less destructive effect than chlorine dioxide, although Lovell (14), in

an examination of fibrous holocellulose from softwoods, indicates that sodium chlorite-acetic acid delignification has little effect on the molecular size of the derived cellulose estimated viscometrically. Claims that oxidation, rather than, or in addition to degradation, takes place have also been made (191). In spite of these difficulties, few modern workers on straw or wood hemicellulose have failed to delignify their samples before extraction, although Anderson (156), Sands (134), and Weihe and Philips (119) are exceptions. These latter workers' observation that the uronic acid content of the extracted hemicellulose increased markedly with strength of alkali might be explained by their failure to delignify before extraction. Under these circumstances, the normal solubility sequence; polyuronide hemicellulose, then cellulosans (128,145) may be reversed (83,91).

Anhydrous liquid ammonia has been used as a swelling agent for wheat straw holocellulose by Bishop and Adams (129). After 36 hours at room temperature in this reagent, it was found that 20.2 % of the holocellulose was soluble in cold water, compared with 3% in absence of ammonia pre-treatment. It was considered by these workers as unlikely that this change would be due to degradation. Later work by Bishop (132), however, showed that anhydrous liquid ammonia could cause appreciable degradation. Extracted polyuronide material showed, in control experiments, a loss of 8.4% after liquid ammonia treatment. Yan (133) has used liquid ammonia as a solvent for lignin in rye straw. A small

concurrent loss of polysaccharide material was recorded.

Other methods used to remove lignin before extraction of hemicellulose include chlorine and 10% ammonia (24); phenol (64), and moist chlorine (22) or bromine. Bromine water has, however, been found ineffective as a delignifying agent for straws (131).

Purification.

Purification of the crude xylan extract was first attempted by Salkowski (88), who introduced the use of Fehling's solution to form the copper complex. The insoluble complex may be decomposed with cold, dilute acid, and the subsequent addition of alcohol then causes the hemicellulose to be precipitated as a white, flocculent mass. Some workers have used gaseous hydrogen chloride to decompose a suspension of the complex in alcohol (115,116,124). Salkowski originally claimed that arabans did not yield such a complex, but later (89) altered this to say that the complex which did form was preferentially soluble in excess of the copper reagent. Heuser (122) has suggested $2C_5H_8O_4$: Cu: 2Na for the composition of the complex. Johnson (77) records that wood xylan yields a complex with basic lead acetate. The use of metallic complexes as a means of purification has been followed by many later workers. Copper sulphate-glycerol was introduced by Norris (27) as an alternative to Fehling's solution, with the claim that the former caused more complete precipitation of the polysaccharide. Recent workers have obtained (140,141) after repeated purification as

the copper complex, xylans whose hydrolysate was free from arabinose, from a crude extract in which the araban content was 7-8%. In constitutional studies of xylan, the removal of accompanying araban has constantly proved a source of difficulty. This fact has led to much controversy, which will be reviewed later, on the possible role of arabinose in the xylan molecule. It may be noted that no wheat straw xylan has yet been obtained whose hydrolysate is free from arabinose residues. Wood hemicelluloses have occasionally been reported with accompanying arabinose residues. An example is the purified xylan from apple wood, in which hydrolysate xylose and arabinose were found in a ratio 7:1 (90). American white oak also yields a quantity of araban (92-95).

Following Schryver's work (92,103(on beechwood hemicellulose, many workers have utilised fractional precipitation methods as a means of separating mixtures of polysaccharides, obtained in alkaline extracts. Fractionation is usually by acidification of the extract and removal of precipitated matter as "hemicellulose A", followed by further precipitation of the filtrate with alcohol or acetone. The latter precipitate, termed "hemicellulose B", has usually been found to be the richer in uronic anhydride (105,134,159). O'Dwyer and Schryver (92), using this method, separated beech hemicellulose into two fractions, A, and B. They showed these to be essentially different, since the

hemicellulose A, alone, contained xylose. Hemicellulose A was more resistant to acid hydrolysis than the B fraction and, in addition, contained 11% glucuronic acid. Fraction B contained 63% galacturonic acid, together with some galactose and arabinose. More elaborate fractionation techniques have been suggested, principally by Norris and Preece (25), whereby the precipitation is accomplished in three fractions. The filtrate, after removal of hemicellulose A, is treated with ½ volume and 1 volume of acetone to precipitate the B and C fractions respectively. The three main fractions are purified by reprecipitation, and further fractionated in A_1, A_2, B_1, B_2, etc. fractions with Fehling's solution, the subscript "1" denoting that the fraction forms an insoluble copper complex. The value of the additional fractionations as a means of separating chemically distinct components is doubtful, and opinion has been expressed that mere separation into portions having varying average chain length is in this way effected (100), particularly as, in some cases, what appears to be a single polysaccharide is precipitated in more than one fraction. The differing physical constants of fractions, the different units present, and the complete absence of some fractions, with certain hemicelluloses (25,47,48,49), however, support the claim that separation of a mixture of polysaccharides is, in most cases, approximated. The extended fractionation technique has been used on number of hemicelluloses containing xylan (47,48,49),

the xylan being found in the A_1, B_1, and C_1 fractions, and having uronic anhydride content increasing in that order. This latter fact may be due to a lower degree of polymerisation (D.P.) and may not, itself, be indicative of a change in molecular species (105). In experiments on wheat bran, Norris and Preece (25) separated a hemicellulose extract containing xylose, arabinose, glucose and uronic anhydride residues into fractions whose hydrolysates contained xylose and uronic anhydride (B_2), arabinose and uronic anhydride (C_2), xylose and arabinose (A_1), and glucose (B_1). No C_1 fraction was present. Similar results have been found with other xylan containing hemicelluloses. The whole of the hemicellulose of boxwood (49), extracted with cold sodium hydroxide solution, was precipitated in the B_1 fraction and contained xylose and uronic acid residues only. Preece (47), examining maize cobs found a xylose uronic acid polysaccharide precipitated in all three fractions A_1, B_1, and C_1, the uronic anhydride content being 3.7%, 5.2%, and 7.4% respectively. This may have been due to the fact that the original extract was a mixture of two or more polyuronides, or a polyuronide and a cellulosan (xylan), with the cellulosan being selectively precipitated in the earlier fractions. A similar observation has been recorded in the case of xylan from oat hulls (106), although a cellulosan such as xylan might be expected to be

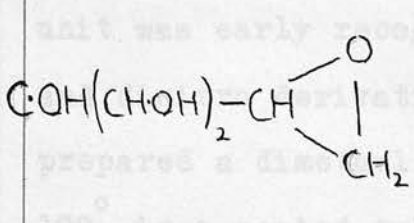
precipitated in the B_1 rather than the A fraction (105). An alternative suggestion is that material incompletely precipitated in one fraction was carried over to the next. This theory finds support in the work of Angell and Norris (27). They report that there is an optimum pH for maximum yield of maize cob, and hop flower, hemicellulose A. At acidities other than this, appreciable quantities may be left in solution.

From a consideration of the above evidence it is apparent that no method of fractionation yet exists ensuring the separation, into its components, of an extract which comprises a mixture of polysaccharides. The separation of polyuronide xylan from xylan as cellulosan, in the extract, is a very real problem. Adams and Castagne have attempted to remove polyuronide material from wheat straw holocellulose with water, leaving cellulosan xylan to be extracted later with dilute alkali (128). Their partial success is in contradiction to the results of Mitchell and Ritter (135), who found the uronic acid content to be higher in the alkaline, than in the aqueous extract of maple wood holocellulose. Perlin (102) has applied fractionation techniques to the derived acetates, and followed the efficacy of the fractionation by deacetylation, hydrolysis, and paper chromatography. The crude acetates were extracted with excess acetone or chloroform, and the extract fractionally precipitated with light petroleum. By such a method, Perlin has succeeded in separating crude water soluble pentosans from wheat flours, into fractions of pure

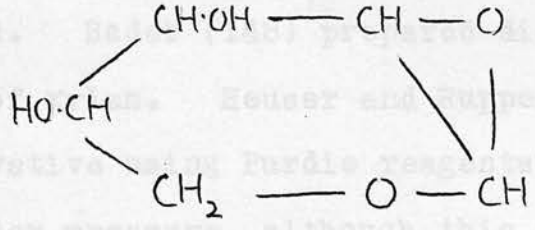
pentosan. Subfractionation in the same manner yielded portions having differing xylose: arabinose ratios, the relative amount of arabinose increasing regularly, in all cases examined, from the less to the more soluble fractions. Perlin has taken the above evidence to show that there exists, in wheat flour, soluble pentosans comprised of a series of arabinose-xylose containing polysaccharides, there being varied composition and structure within the series. Fractional precipitation of the acetates from chloroform solution has also been followed by Adams and Castagne (130), investigating a hot water soluble polyuronide from wheat straw. The crude hemicellulose contained xylose, arabinose, glucose, galactose and a hexuronic acid. After fractionation, a pentosan fraction containing only xylose and arabinose was isolated.

Constitution.

It was realised early in the history of xylan, that D-xylose was the fundamental residue, since this sugar was obtained on mild acid hydrolysis (74,88,136). It was not so early realised, however, that the substance might be a long chain polymer. Among early formulae suggested were those of Tollens (I) and (II)



(I).

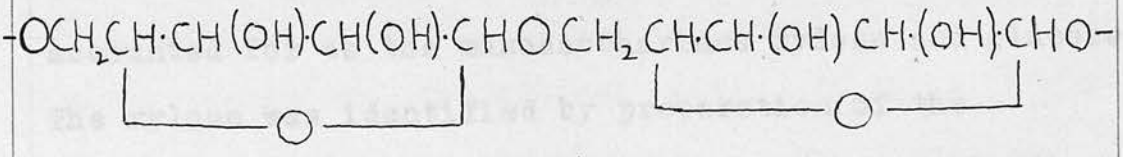


(II).

Much controversy centred on early attempts to establish an empirical formula. Thomsen (79), Koch (136), and Wheeler and Tollens (74,120), gave results approximating $C_6H_{10}O_5$. Johnson (77), after stringent drying precautions, found $C_5H_8O_4$ for xylan from corn (Zea mays) cobs, and $C_4H_6O_3$ for beech xylan. He showed that air dry xylan contained about 8% water. Later workers have put this figure even higher (108). Schorsch (109), following an earlier suggestion by Salkowski, advanced $C_{10}H_{18}O_9$ as more nearly in accord with the experimental evidence. Heuser and Schorsch (107) claimed that under certain conditions xylan retains sodium hydroxide to give a compound $\left[(C_5H_8O_4)_2\ Na\cdot OH\right]_n$. However the sodium hydroxide is not firmly held, and may readily be eliminated. The point was finally settled by Link (121), who analysed 15 highly purified xylans from various sources by the Pregl micro method. He arrived at the empirical formula $(C_5H_8O_4)_n$, and the conclusion that previous conflicting results were due to the difficulty of obtaining xylan free from moisture. A little previously, analyses of esparto xylan based on simultaneous C.,H., and moisture determinations had obtained the same result (108).

The presence of two free hydroxyl groups per xylose unit was early recognised. Bader (148) prepared diacetyl and dinitro derivatives of xylan. Heuser and Ruppel (146) prepared a dimethyl derivative using Purdie reagents at 100° in a sealed tube under pressure, although this method

was criticised by later workers (108). From a study of the hydrolysis products of rice and wheat xylan methylated by a modification of Heuser and Ruppel, Komatsu (147) put forward the constitution I. The resulting dimethyl xylose was identified as 2:3 dimethyl xylose by oxidation to dimethoxy-glutaric acid. Such a formulation had been indicated earlier by Komatsu and Kashima (149) investigating the hydrolysis products of xylan and its acetate.



Criticism of this work was advanced by Hampton, Haworth, and Hirst (108) who suggested that the dimethyl xylan claimed by Komatsu, Inoue and Nakai was probably a mixture of degraded products. They showed that the methylation procedure followed by these workers led to a low melting syrup having a dextro rotation. Hydrolysis of the syrup afforded a quantity of trimethyl xylose in addition to the dimethyl sugar. The failure of Komatsu and his co-workers to characterise the dimethyl fragment, which they identified with 2:3-dimethyl xylose, was also noted by Hampton, et al.

In order to show that the pentose present in esparto holocellulose, then termed "esparto cellulose," hydrolysate had origin in a xylan, and not a polysaccharide possessing both hexose and pentose residues, a series of experiments were carried out by Irvine and Hirst (22). Esparto "cellulose" was converted quantitatively into its acetate. The resulting mixture of hexosan and pentosan acetates was then converted to the corresponding methyl glycosides under conditions which were shown, by control experiments, to cause minimum loss of pentose. By this means, 92% of the theoretical quantity of esparto "cellulose" could be accounted for as the monosaccharides xylose and glucose. The xylose was identified by preparation of the characteristic trimethyl β methyl xyloside. No other sugars were detected in the hydrolysate. The xylose/glucose ratio obtained was not constant but varied with the pretreatment given to the "cellulose." Although, under certain conditions, cellobiose could be isolated, from the true cellulose after removal of the pentosan, no disaccharide possessing both xylose and glucose residues could be obtained from the unextracted esparto "cellulose."

From these considerations, and the fact that a xylan, which on hydrolysis yielded only xylose, could be extracted from the esparto "cellulose" with boiling dilute alkali, Irvine and Hirst concluded esparto "cellulose" to be a mixture of two polysaccharides - the hexose, which comprised the true cellulose of the

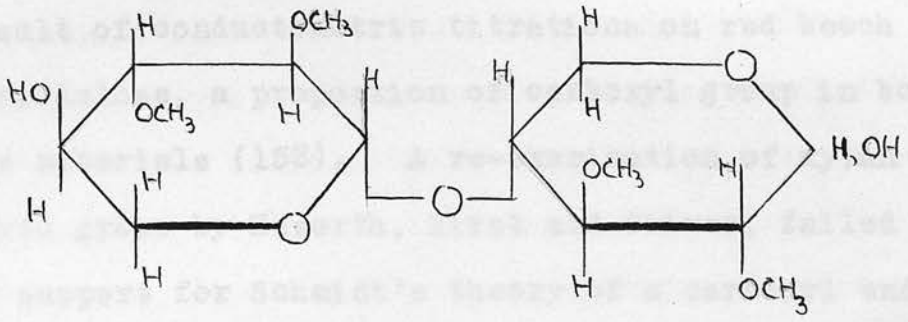
material, possessing only glucose residues, and the admixed pentose, only xylose. They suggested that the two polysaccharides were probably associated in solid solution.

Further work on esparto xylan was undertaken by Hampton, Haworth and Hirst (108). They supported the view that xylan contained only xylose units, obtaining 93% of the theoretical yield of crystalline xylose on hydrolysis with dilute nitric acid. The difficulty, noted by Heuser and Ruppel (146), of achieving complete methylation using dimethyl sulphate and sodium hydroxide was avoided by substituting potassium hydroxide. By this modified procedure, xylan was completely methylated in two operations, the product having $[\alpha]_D^{20}$ - 92° in chloroform, and being non reducing. The methylated product was hydrolysed with methanolic hydrogen chloride to a dimethyl methyl xyloside in 90% yield, identified as the 2:3-dimethyl methyl xylopyranoside. The identification was based on a number of considerations. The dimethyl methyl xyloside was further methylated and hydrolysed to give crystalline 2:3:4 - trimethyl D-xylopyranose, previously prepared by Hirst and Purves (150), and others (151). Hydrolysis of the dimethyl methyl xyloside gave the free dimethyl sugar, which afforded a crystalline anilide. On oxidation of the dimethyl xylose with bromine water, a syrupy lactone was prepared from which crystalline phenylhydrazide and p. bromphenylhydrazide derivatives of the corresponding acid were obtained. The phenylhydrazide differed from the known phenylhydrazide

of 3:5 - dimethyl xylonic acid (152). The rate of hydrolysis of the above lactone, and its further methylation to the known 2:3:5 - trimethyl-γ -xylono lactone (152) were in agreement with the substance being a γ lactone. This indicated that C_4 in the original dimethyl methyl xyloside was free. Finally, the failure of the dimethyl sugar to give an osazone pointed to the existence of a methyl ether group on C_2. These findings proved the original dimethyl methyl xylopyranoside to be the 2:3-isomer. No other dimethyl methyl xylosides were isolated from the hydrolysis products of the methylated xylan. The conclusion drawn by Hampton, Haworth and Hirst was that carbons 4 and 5 of the xylose residues were involved in ring formation, and in mutual linking of residues in the polysaccharide. The precise allocation of these functions was left unsettled, but it was pointed out that stability considerations would favour a pyranose structure, and thus a 1:4 - link between units. The high laevo rotation of the xylan was also noted as indicative of a β link. The structure suggested for the polysaccharide was thus closely similar to the known structure of cellulose with the terminal CH_2OH group replaced by H. No indication of chain length was given.

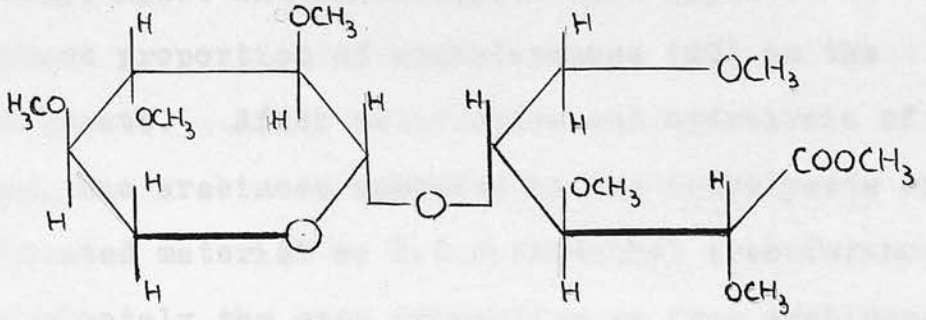
That xylose residues were present in xylan in the pyranose form was finally established by Haworth and Percival (110) again using Esparto xylan. Methylated xylan was degraded by acetolysis at 0° C. for a short interval. A partly methylated disaccharide was isolated

and shown to have the structure (I).



I

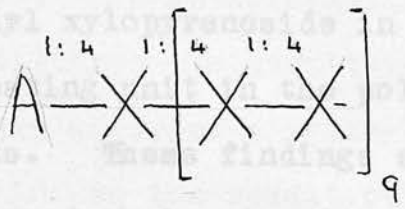
The products of acetolysis were first deacetylated and then oxidised with bromine water. The disaccharide gave a partly methylated xylobionic acid which, on further methylation and hydrolysis yielded the methyl ester of hexamethyldixylobionic acid (II).



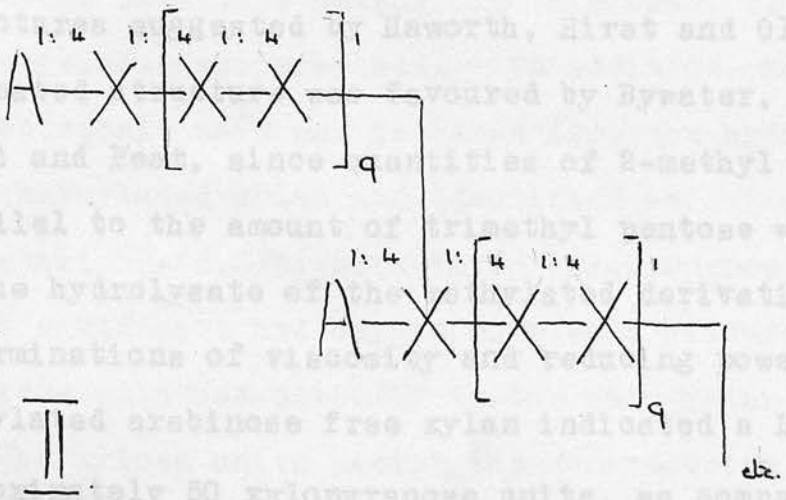
On hydrolysis the latter substance afforded crystalline 2:3:4-trimethyl xylopyranose and 2:3:5-trimethyl-γ-xylonolactone, in yields of 80% and 73% respectively. The 2:3:5-trimethyl-γ-xylonolactone, which had previously been prepared by methylation, hydrolysis and oxidation of dimethyl xylose monoacetone by Haworth and Porter (152), was characterised as the crystalline phenylhydrazide of 2:3:5-trimethyl xylonic acid. This evidence placed the xylopyranose structure of xylan on a secure basis, and showed that linking between contiguous xylan residues in the polysaccharide must be 1:4.

In the following year, 1932, Schmidt reported, as a result of conductometric titrations on red beech xylan and cellulose, a proportion of carboxyl group in both of these materials (153). A re-examination of xylan from esparto grass by Haworth, Hirst and Oliver, failed to find support for Schmidt's theory of a carboxyl end group. Evidence was found, however, in favour of the view that the xylan chain was terminated by an L-arabofuranose residue. The fact that, under the most careful conditions, the highest amount of xylose previously obtainable on hydrolysis had been 93%, supported this. Examining various samples of xylan, Haworth, Hirst and Oliver found what appeared to be a constant proportion of arabofuranose (6%) in the hydrolysate. After methylation and hydrolysis of the xylan, the arabinose appeared in the hydrolysate of the methylated material as 2:3:5-trimethyl arabofuranose in approximately the same proportion as free arabinose had been detected in the hydrolysate of the unmethylated polysaccharide. No partly methylated arabinose or fully methylated xylose was detected. The 2:3:5-trimethyl L-arabofuranose was characterised by preparation of the corresponding crystalline lactone, from which the crystalline 2:3:5-trimethyl arabonamide was obtained. Quantitative separation of the trimethyl arabinose was accomplished by careful fractional distillation of the glycosides formed on methanolysis. On the basis of these results, the repeating unit of esparto xylan was

assumed to be 1 arabofuranose to every 18 to 20 xylopyranose residues. Some 5% of unidentified monomethyl xylose was also separated from the hydrolysis products of the fully methylated xylan, the authors expressing the view that this could have structural significance, or could be due to undermethylation. Measurements of viscosity of the methylated xylan in m-cresol gave a value corresponding to an average of 75-80 C_5 units. Possible structures advanced for the xylan molecule were a straight chain (I), a laminated structure (II), a branched (~~III~~), or looped (IV) structure. The straight chain hypothesis assumed the monomethyl xylose to have no structural significance, whilst the looped possibility took into account the fact that no evidence had been obtained by these workers for the existence of a free reducing group in xylan, (221).

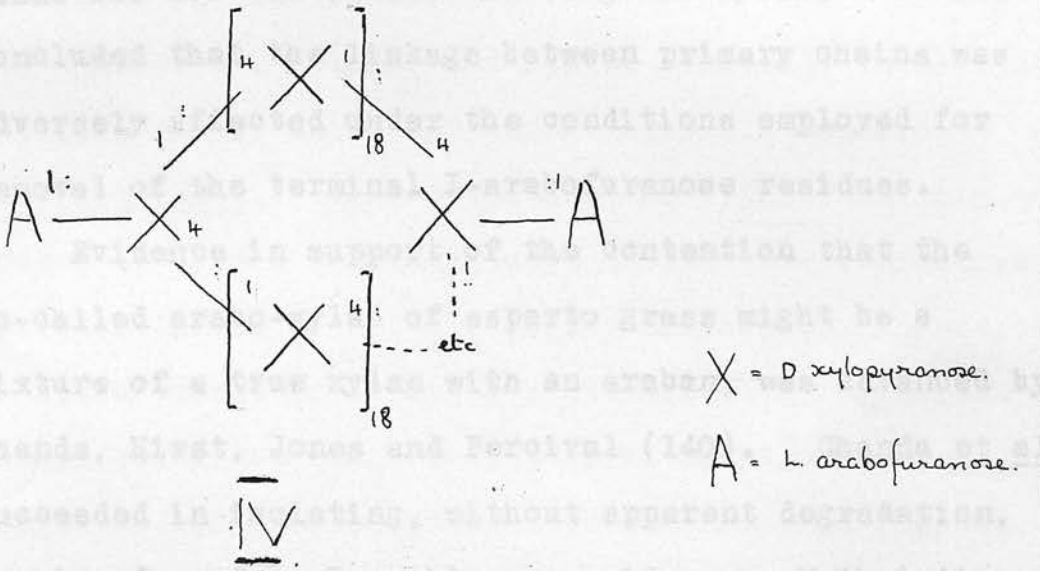


I.



II

see overleaf.



IV

In an attempt to limit the structures possible for the xylan molecule, work on esparto xylan was again taken up by Bywater, Haworth, Hirst and Peat in 1937 (139). By treatment of the arabinose containing xylan with 0.2% aqueous oxalic acid at 100° C., complete removal of the arabinose residues was effected without appreciable degradation of the xylan chain. The methylated arabinose free xylan gave, on hydrolysis, 2:3:4-trimethyl methyl xylopyranoside in a yield corresponding to a repeating unit in the polysaccharide of 18-19 xylopyranose units. These findings eliminated the possibility of a ringed chain structure (IV). Of the remaining possible structures suggested by Haworth, Hirst and Oliver, a laminated structure was favoured by Bywater, Haworth, Hirst and Peat, since quantities of 2-methyl xylose parallel to the amount of trimethyl pentose were identified in the hydrolysate of the methylated derivative. Determinations of viscosity and reducing power of the methylated arabinose free xylan indicated a D.P. of approximately 50 xylopyranose units, as compared with a

value for D.P. of 91 for the original xylan. It was concluded that the linkage between primary chains was adversely affected under the conditions employed for removal of the terminal L-arabofuranose residues.

Evidence in support of the contention that the so-called arabo-xylan of esparto grass might be a mixture of a true xylan with an araban, was advanced by Chanda, Hirst, Jones and Percival (140). Chanda et al succeeded in isolating, without apparent degradation, a xylan free from L-arabinose residues. Methylation studies indicated a singly branched molecule containing approximately 75 β D-xylopyranose units, joined by 1:4-linkages. This conclusion was supported by the results of periodate oxidation, and by reducing power and osmotic pressure determinations. Subsequent work, however, has emphasised the possibility that an arabo-xylan may also be present in espart hemicellulose (67). An investigation by Chanda, Hirst and Percival (141) into the structure of a xylan from pear cell wall again afforded a xylan free from arabinose residues. Again, on the result of methylation studies, a singly branched structure having, in this case, 115 β D-xylopyranose residues was proposed. In addition, however, an aldobiuronic acid was isolated from the hydrolysate of the methylated xylan and identified as 2-methyl 3-(2:3:4:-trimethyl ∝ D:glucuronosido) D-xylose. This could have had origin only in a glucuronic acid residue attached directly to the main xylan chain on any of the xylose units except the non-reducing terminal

residues, or the branching point.

Mc.Ilroy, Holmes, and Mauger (142,143) investigated a xylose-uronic acid containing hemicellulose from Phormium tenax. They concluded the xylan from this source to have a repeating unit of 9-10 D-xylopyranose residues united by 1:4 glycosidic linkages. In addition, an aldobiuronic acid having a glucuronic acid residue linked to xylose, was considered to be attached to some single xylose residue other than the non-reducing end group. In support of this, an aldotriuronic acid resistant to methanolysis and comprising residues of 3-methyl D xylose, 2:3-dimethyl D xylose, and 2:3:4-trimethyl D glucuronic acid was identified in the hydrolysate of the methylated xylan. The precise state of aggregation of the molecule was left unsettled.

In a recent examination of water soluble pentosans from wheat flour, Perlin has presented evidence to show that these polysaccharides consist of a straight chain of 1:4 β linked D-xylopyranose units with L-arabofuranose residues attached, as side chains, through positions 2 or 3 of approximately every second xylose residue. His evidence is based on graded hydrolysis, methylation, and periodate oxidation. The further observation, that on progressive removal of L-arabofuranose residues by graded hydrolysis the residual polysaccharide becomes less water soluble, has prompted the suggestion by Perlin, that a mechanism for the addition or removal of arabinose units from a xylan "core" may exist in the plant. Such

a mechanism would allow the translocation of soluble araboxylan, and deposition of this as an insoluble xylan. It would also imply a relationship between pentosans distributed throughout the plant.

Weihe and Philips (119), investigating a wheat straw hemicellulose, found xylose, arabinose, and a uronic acid in the hydrolysate in the ratio 23.0;0.9;1.0. The uronic acid was separated as the barium salt but was not definitely identified. Bishop and Adams (129) fractionated hemicelluloses extracted from wheat straw under varying conditions. Analysis of the isolated fractions showed some systematic differences, with pentosan contents increasing, and uronic acid decreasing, progressively, in the more alkali soluble fractions. Hydrolysis of the different fractions revealed an unexpected similarity of composition. Xylose, arabinose, glucose, galactose and a hexuronic acid were found in all hydrolysates in the approximate molar ratio 40:7:2:1:4. A uronic acid, resistant to mild hydrolysis, was isolated as the barium salt. This, on further vigorous hydrolysis, gave xylose and a hexuronic acid unit identified by its R_G value as galacturonic acid. A methoxyl/uronic anhydride ratio of unity, found on analysis of the barium uronate, however, made it probable that the hexuronic acid residue carried a single methyl ether group.

Adams and Castagne (128) showed that crude hemicellulose extracts of wheat straw could be fractionally

precipitated into fractions containing varying amounts of uronic acid. For several fractions the molar ratio found for methoxyl/uronic anhydride was approximately unity. This, however, may have been fortuitous since in other fractions the methoxyl content was excessively high, and could be explained only by assuming that part had origin in residual lignin. Intrinsic viscosity measurements of the acetates indicated that all hemicellulose fractions had a D.P. of 25-30. Xylose, with some arabinose and glucose were present in the hydrolysates of all fractions and galactose in the hydrolysate of the water soluble extracts only. The uronic acid was tentatively identified as a monomethoxyl galacturonic acid linked to a xylose residue. Identification was afforded by the R_G values of the units released after vigorous hydrolysis and consequent demethylation of the acid residue.

A further examination of the hemicellulose extracted from wheat straw by hot water has been made by Adams and Castagne (130). A fraction containing pentosan and uronic acid only was obtained, although fractions of pure hexosan were not isolated. The authors considered, however, that hexosan and pentosan were not chemically combined. Instances of hexosan in combination with xylan have been claimed from a number of sources (93,94,137,155,156.) although possible glucosan or cellulose contamination has not always received adequate consideration. Adams and Castagne record also that although the constancy of the xylose/arabinose ratio

found on hydrolysis of the various fractions was strongly indicative of chemical union, the possibility of a mixture of xylan and araban could not be excluded. Support for the latter possibility has been advanced by Boggs, Ehrenthal, Smith et al (137). These workers examined methylated corn cob and wheat straw xylan and showed that arabinose units in addition to those occupying terminal positions were present. Trimethyl xylose was also identified in the hydrolysate of the methylated material. Ehrenthal and Smith (138) have also recorded that after periodate oxidation of "xyloaraban," unoxidised arabinose units may be detected. They take this to indicate that these units cannot function as "end" group in the polysaccharide, and are more likely to be present as highly branched araban.

A more detailed study of the constitution of an arabinose rich xylan from wheat straw was reported by Adams (98) shortly after the completion, by the author, of the present work on straw hemicellulose. The hemicellulose examined by Adams contained L-arabinose 13.6%; D-xylose 71.7%; and uronic anhydride 10.6%. Graded hydrolysis with dilute oxalic acid preferentially removed arabinose residues, leaving a xylan to which the uronic acid units originally present remained attached. Maximum removal of arabinose residues with minimum removal of xylose was obtained with a 2 hour heating period. Under these conditions 92% of the total arabinose, and 3.5% of the total xylose were obtained in the hydrolysate, the free sugars being separated by

paper chromatography and estimated by the micro method of Somogyi (123). Complete methylation, followed by fractionation and methanolysis of the undegraded hemicellulose yielded a mixture of sugars. After the methanolysis mixture had been treated with barium hydroxide to convert the methylated uronic acid methyl ester present to the corresponding barium salt, the methyl glycosides were separated by solvent extraction. Trimethyl pentosides were removed with n.-pentane; dimethyl methyl xyloside with diethyl ether, and mono methyl xyloside with chloroform.

The trimethyl pentosides were partially hydrolysed with hydrobromic acid under conditions ensuring that the arabinoside was completely hydrolysed whilst the xyloside remained unaffected. A further hydrolysis using hydrochloric acid then accomplished the hydrolysis of the xyloside. Analysis of the reducing power at the end of the partial and the complete hydrolysis periods indicated the composition of the trimethyl pentose fraction to be 2:3:4-trimethyl - D-xylose, 2.7%; 2:3:5-trimethyl-L-arabinose 12.4%. The trimethyl-D-xylose was identified in its characteristic crystalline form, the trimethyl-L-arabinose being converted to the crystalline 2:3:5-trimethyl-L-arabonamide.

The monomethyl pentoside was, on hydrolysis, identified as 2-monomethyl-D-xylose. No mono or dimethyl arabinoses were detected.

Estimation of the ratio of methylated sugars present in the hydrolysate of the methylated material gave a molar

ratio of 5:25:1:5 for 2-monomethyl-D-xylose; 2:3-dimethyl-D-xylose; 2:3:4-trimethyl-D-xylose; 2:3:5-trimethyl-L-arabinose. This indicated that for every xylose residue which represented a branching point there appeared an arabinose end group. The methylated aldobiuronic acid present in the hydrolysate was identified by reduction and hydrolysis as 2-methyl 3-[2:3:4-trimethyl ∝ D-glucuronosido]-D-xylose.

On the basis of the above evidence, Adams has proposed a structure for the hemicellulose consisting of 32 D-xylose residues linked β 1:4- in a chain to which five L-arabinose residues and three D-glucuronic acid units are attached, as side groups, by 1:3 glycosidic bonds. Results of periodate oxidation experiments were found to support a branched structure of this nature.

Bishop (132) examined a hemicellulose extracted from wheat straw holocellulose with anhydrous liquid ammonia. He showed the hydrolysate to consist of xylose, arabinose, glucose, galactose and uronic anhydride in the ratio 11:3:3:1:2.5. No fractionation of the extract was attempted. In a separate investigation, the uronic acid was identified as glucuronic acid. Methoxyl determinations gave a value which could be explained by assuming that one third of the uronic acid residues present carried a methyl ether group.

Wood hemicelluloses.

The hemicellulose of American white oak has been examined by O'Dwyer (92). She concluded that at least two polysaccharides were present - an araboxylan and a

galactomannan. The xylose/arabinose ratio was estimated at 51.5:18.5. An investigation by O'Dwyer of the hemicelluloses of English oak indicated that sapwood xylan differed from heartwood xylan in possessing combined glucose residues (93,94,95.). The sapwood hemicellulose on prolonged hydrolysis with takadiastase or mild hydrolysis with mineral acid was completely fractionated into a "soluble polysaccharide" in the ratio of 2:3 with xylose. The "soluble polysaccharide" on further hydrolysis yielded a monomethyl hexuronate and xylose in the ratio 1:6. O'Dwyer considered it likely that oak heartwood hemicellulose A contained a repeating unit of $(xylose)_{11}$ - monomethyl uronic acid, and that sapwood hemicellulose A contained an additional combined glucose residue. Similar results were indicated for the B fraction from the same source. A basic repeating unit of $(xylose)_6$ - monomethyl uronic acid was postulated for oak heartwood hemicellulose B, this being identical with the proportions of the hydrolysis resistant nucleus from hemicellulose A preparations. The existence of polyuronide-pentosan having combined anhydroglucose units is of interest in the question of the derivation of hemicelluloses from starch. Preece (47,48.) obtained a hemicellulose fraction from boxwood in which the repeating unit also appeared to be xylose:uronic acid in molar ratio 6:1.

Sands and Gary (134) examined mesquite wood hemicellulose extracted with cold, dilute alkali before delignification. Fractions having uronic acid content

10-18% were separated. Sands and Gary supposed the extract to be composed of two hemicelluloses, one having a repeating unit $(xylose)_{11}$ - uronic acid and the other $(xylose)_{6}$ - uronic acid. "Body X", an insoluble product of acid hydrolysis (158) later shown to be formed by condensation of lignin with pentose decomposition products, was supposed by Sands and Gary to form an integral part of the hemicellulose. Later work by Sands and Nutter (24) in which mesquite wood hemicellulose was extracted under more vigorous conditions, gave fractions whose composition varied from xylose:monomethyluronic acid (7:1) to xylose:monomethyluronic acid (15:1). It has been suggested (157) that the extraction method employed would cause considerable quantities of xylan of cellulosan origin to accompany the polyuronide. This contention is supported by the fact that the presence of an easily hydrolysable glucosan was demonstated in one of the fractions.

Anderson et al (155) examined mixtures of hemicelluloses present in extracts of a number of hardwoods before and after delignification. Each extract was shown to contain a xylan of composition I, x varying from 10 to 19.

(Monomethyluronic acid) - $(xylose)_{x}$

I

The acid residue was not characterised. The hemicellulose from the sapwood of two woods examined - black locust and lemon wood - extracted before delignification gave a hydrolysate in which glucose, xylose and a

uronic acid were present. This was attributed to a glucosan-xylan polyuronide, since the glucose residue could be removed from the polysaccharide with enzymes only with difficulty. However, since starch was shown to be present in the hot water pre-extraction of these two lignified woods, the postulated glucosan-xylan polyuronide may well have comprised a mixture of xylan polyuronide and glucosan.

Anderson, Kesselman and Bennet (156) extracted the sap and compression wood of white pine (Pinus Strobus L.) before and after delignification. They suggested that a hemicellulose was present having the constitution II, and that the occurrence in the hemicellulose of a mannan free from uronic acid was due to the breaking away, under certain conditions, of the anhydromannose units as a block. No indication of the state of aggregation was given. A similar structure, in which the hexose units attached to xylose were glucose residues, was earlier advanced by O'Dwyer for oak sapwood hemicellulose (93,94,95,112.).

(Monomethyluronic acid) - $(\text{xylose})_n$ - $(\text{mannose})_m$

II

The present work on straw and beech hemicelluloses was undertaken in order to ascertain what further light could be thrown, by the application of recently developed techniques, on the structure of the xylan-polyuronides present in these materials.

NOTES.

1. Methoxyl determinations were performed by the volumetric method of Vieböck and Brecher (197,198, 199) unless otherwise stated.

2. Where chromatogram solvent mixtures were two-phase, the upper phase was used for elution of the paper.

3. Melting points taken were uncorrected, and were performed on a Kofler micro-melting point apparatus.

EXPERIMENTAL.

The wheat (Variety, White Victor) was planted at Shothead Farm, Balerno, in October 1949, and cut September 1950.

Preparation of holocellulose and xylan.

(a) Small scale.

A sample of straw (10 g.) was cut manually into pieces 3 - 5cm. long and these were extracted in a Soxhlet apparatus with benzene (200 ml.), and methanol (200 ml.) to remove, respectively, wax and colouring matter. The dry residue was then suspended in 400 ml. of water containing glacial acetic acid (50 ml.), sodium chlorite (50 g.) and sodium acetate (2 g.). The temperature was maintained at 30° for 24 hours, after which time the solution was dark brown in colour, and at pH 8. The residue was washed repeatedly with cold water and then with acetone, finally being air dried (6.5 g.).

The dry white holocellulose, which retained the structure of the original straw, was extracted with sodium hydroxide solution (200 ml.;4%) on a mechanical shaker for 40 hours. The resulting brown slurry was filtered through a coarse glass sinter, and the solid residue was re-extracted with fresh sodium hydroxide solution (200 ml.;4%). The solutions obtained from both extractions were combined, acidified with glacial acetic acid and the xylan was precipitated by addition of the extract to methanol. A flocculent precipitate was obtained which was separated at the centrifuge, washed with aqueous ethanol (50:50) until acid free,

then with ethanol and finally with ether. The light brown solid was dried in air and over phosphorus pentoxide in a desiccator, 2.1 g.; $[\alpha]_D^{20}$ - 79.1° (C, 0.4 in 0.2N sodium hydroxide).

Chromatographic examination of the hydrolysate (0.5N sulphuric acid; 100° ; 6 hours) on a Whatman No.1 filter sheet in butanol: benzene: pyridine: water (5:1:3:3:) for 44 hours indicated, on development with saturated aniline oxalate solution, xylose and arabinose to be the only two sugars present, the latter being approximately 5% of the total. No glucose, galactose or uronic acid was detected. Relevant standard sugar samples were run on the same paper.

Attempted purification by fractional precipitation.

The crude xylan was dissolved in sodium hydroxide solution (50 ml.;4%), acidified with glacial acetic acid and fractionally precipitated using (a) half, (b) one, (c) five volumes of acetone. A sample was fractionally precipitated in a similar manner in ethanol. Paper chromatographic examination of the hydrolysates indicated that no separation of xylose from arabinose had been accomplished. Precipitation was found virtually to be complete on addition of to ½ volume of acetone.

Purification by Copper Complex formation.

The xylan was dissolved in sodium hydroxide solution (50 ml.;4%) and treated with an equal volume of freshly prepared Fehling's solution to give the blue, gelatinous copper complex. This was filtered

through a pad of muslin and washed with Fehling's solution, followed by cold water to which a little alkali had been added. The blue complex remaining was then dispersed in water, and decomposed by the addition, to it, of cold hydrochloric acid (2N). A clear solution was obtained, the final acidity of which did not exceed 0.5N. After standing at room temperature ($\frac{1}{2}$ to $\frac{3}{4}$ hour) the polysaccharide was regained by addition of the acid solution to ethanol (1 vol.). A flocculent white precipitate was thus obtained, which was separated at the centrifuge and washed repeatedly with alcohol:water mixture (50:50) to which a trace of hydrochloric acid had been added, to remove copper. Washing with neutral alcohol:water mixture (50:50) then followed, to remove acid. Finally, the precipitate was washed with absolute ethanol and then ether, and air dried. $[\alpha]_{\ell}^{17}$ - 89.8° (c, 1.0 in 4% sodium hydroxide) All washings were performed by brisk agitation of the solid with the wash liquid, followed by removal of the liquid at the centrifuge.

An attempt to employ glacial acetic acid to decompose the copper complex was unsuccessful, since the solution at this stage is heavily buffered. A light blue, colloidal precipitate resulted, even on addition to the complex of a large excess of acetic acid, and after standing at room temperature for 12 hours. On addition of ethanol (1 vol.) to this solution, a blue-white precipitate was obtained which suggested that the complex had not been fully decomposed. After washing with ethanol:water (50:50), ethanol, and ether, successively in the usual manner, and air drying, the

resulting solid was only partially soluble in sodium hydroxide solution (4%). Paper chromatographic examination of a sample hydrolysate showed an arabinose/xylose ratio identical with that obtained from a polysaccharide sample of the hydrochloric acid decomposed complex.

(b) Large scale.

Chopped wheat straw (500 g.) was defatted, and delignified, the following modifications in the previous procedure being made:

The concentration of acetic acid present at delignification was increased. The proportions used were, water (5 l.), glacial acetic acid (500 ml.), sodium chlorite (1400 g.), and sodium acetate (60 g.). The crude alkaline extract was acidified with acetic acid and precipitated in ethanol (1 vol.). Before acidification the crude extract was filtered, in turn, through a sintered glass funnel, filter paper (Whatman No.42), and a well washed bed of "filter cel." Without such clarification, a component remained which gave a colourless, gelatinous precipitate on dissolution of the dry, crude polysaccharide in sodium hydroxide solution. The gelatinous precipitate was insoluble in cold sodium hydroxide (10N) and, after hydrolysis and examination on a paper chromatogram, showed some glucose, in addition to xylose and arabinose.

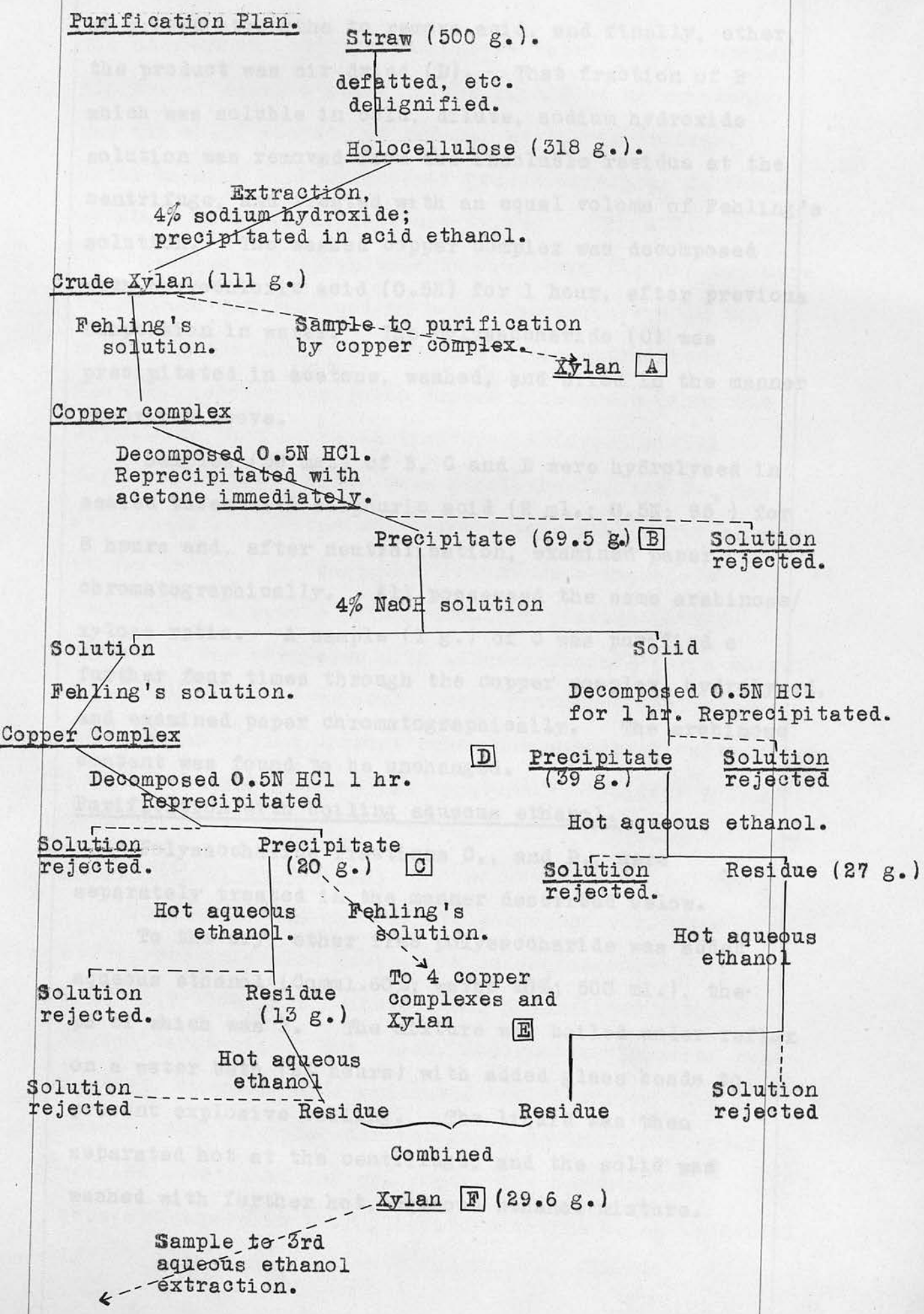
Purification; large scale.

(a) A small sample (1 g.) of crude, dry xylan was purified through the copper complex with Fehling's

solution, as previously described. The recovered polysaccharide (A) was hydrolysed in a sealed tube with sulphuric acid (1N; 95° ; 8 hours), neutralised with barium carbonate and examined on a paper chromatogram, against relevant standards. Visual estimation, by spot size and colour, indicated a slight reduction in the arabinose:xylose ratio which was confirmed by a quantitative estimation by periodate (11). This indicated a fall in percentage arabinose from 6.2% (crude) to 5.4% (xylan A), calculated on the basis of 100% total pentose (see p.47).

(b) The main bulk of crude xylan was treated according to the procedure outlined in the plan, (overleaf).

An attempt was made to purify the polysaccharide on the assumption of varying stabilities of the different copper complexes. It was seen that if the copper complex were dispersed in water (2 - 3 l.), acidified with hydrochloric acid (2N) to approximately 0.5N, and precipitated by the addition of it to acetone immediately full solution had been effected, then the resulting precipitate (B), after washing and drying in the usual manner, was only partially soluble in sodium hydroxide solution (4%). The insoluble portion, a blue paste, was undecomposed copper complex. This was treated with hydrochloric acid (0.5N) for 1 hour, and precipitated in acetone. After repeated washing with acidified acetone (50:50, acetone/0.5N hydrochloric acid) to remove copper and sodium salts, followed by washing with alcohol


Purification Plan.
Straw (500 g.).
defatted, etc.
delignified.
Holocellulose (318 g.).
Extraction,
4% sodium hydroxide;
precipitated in acid ethanol.
Crude Xylan (111 g.)
Fehling's solution.
Sample to purification by copper complex.
Xylan A
Copper complex
Decomposed 0.5N HCl.
Reprecipitated with acetone immediately.
Precipitate (69.5 g.) B
Solution rejected.
4% NaOH solution
Solution
Solid
Fehling's solution.
Decomposed 0.5N HCl for 1 hr. Reprecipitated.
Copper Complex
D
Precipitate (39 g.)
Solution rejected
Decomposed 0.5N HCl 1 hr.
Reprecipitated
Hot aqueous ethanol.
Solution rejected.
Precipitate (20 g.) C
Solution rejected.
Residue (27 g.)
Hot aqueous ethanol.
Fehling's solution.
Hot aqueous ethanol
To 4 copper complexes and Xylan
E
Solution rejected.
Residue (13 g.)
Hot aqueous ethanol
Solution rejected
Residue
Residue
Solution rejected
Combined
Xylan F (29.6 g.)
Sample to 3rd aqueous ethanol extraction.

of graded strengths to remove acid, and finally, ether, the product was air dried (D). That fraction of B which was soluble in cold, dilute, sodium hydroxide solution was removed from the insoluble residue at the centrifuge, and treated with an equal volume of Fehling's solution. The washed copper complex was decomposed with hydrochloric acid (0.5N) for 1 hour, after previous dispersion in water. The polysaccharide (C) was precipitated in acetone, washed, and dried in the manner described above.

Samples (50 mg.) of B, C and D were hydrolysed in sealed tubes with sulphuric acid (2 ml.; 0.5N; 95°) for 8 hours and, after neutralisation, examined paper chromatographically. All possessed the same arabinose/xylose ratio. A sample (1 g.) of C was purified a further four times through the copper complex, hydrolysed, and examined paper chromatographically. The arabinose content was found to be unchanged.

Purification with boiling aqueous ethanol.

Polysaccharide fractions C., and D., were separately treated in the manner described below.

To the dry, ether free polysaccharide was added aqueous ethanol (Comml.60%; water 40%; 500 ml.), the pH of which was 7. The mixture was boiled under reflux on a water bath (24 hours) with added glass beads to prevent explosive boiling. The liquid was then separated hot at the centrifuge, and the solid was washed with further hot, aqueous ethanol mixture.

The extraction under reflux was repeated with a further 500 ml. of aqueous ethanol, the liquid being removed, and the solid washed, as before, at the centrifuge. After washing with alcohol of graded strengths followed by ether, the solids were air dried.

Samples of polysaccharide fractions C., and D., were hydrolysed and examined on paper chromatograms in the normal manner after one, and after two extractions. The arabinose;xylose ratio showed a decrease after one extraction, but a second treatment afforded little further change. After both fractions C., and D., had separately been given two extractions, sample hydrolysates showed both to be identical. They were therefore combined. A sample of the combined polysaccharide (Xylan F) was extracted with aqueous ethanol for a third time, and dried as before. On hydrolysis, and examination of the product paper chromatographically, no further purification was apparent. Yield of Xylan F, 29.6 g.

Quantitative estimations.

Samples (20-25 mg.) of dry polysaccharide were hydrolysed in sealed tubes with sulphuric acid (0.5 ml.; 1N; 99° ; 9 hours), and neutralised with barium carbonate. The clear, supernatant solution was spotted, by means of a graduated capillary, on to a number (3-5) of paper chromatograms so that each paper carried a total of 1-2 mg. sugar, the amounts on separate papers in a single determination being identical.

The measured amounts of sugar solution were contained in the central strips only. The side strips, which were removed and sprayed after development of the paper, in order to fix the position of the sugars, carried the same concentration of sugar as did the central, unsprayed portion. The chromatograms were developed for 48-55 hours with benzene: butanol: pyridine: water solvent. (1:5:3:3), dried in air overnight and in a vacuum desiccator over water (1-2 hours), and cut into pieces corresponding to the position of the sugars as indicated by the sprayed side strips. Clear separation was obtained between xylose and arabinose, and no overlapping was experienced between central and side strips when the width of the latter was 3 cms. In a single determination, the arabinose strips from 3-5 papers were combined, and arabinose present estimated in comparison with the xylose from a single paper. In this way, the disparity between titre figures obtained for xylose and arabinose consequent on the very small quantity of arabinose present, was lessened.

The sugar was eluted from the strips with cold water (164), and estimated using sodium metaperiodate (175). Separate blanks were run, in each determination, for each sugar estimated, the areas of blank being adjusted to equal that of the unknown in each case. Sodium metaperiodate solution (2 ml.; 0.25N) was used and sugar solutions were adjusted to 7 ml. Oxidation time allowed was 20 minutes on the boiling water bath. Neutral ethylene glycol was used, and formic acid was

estimated against carbonate free sodium hydroxide (0.01N). Blanks did not exceed 0.06 ml.

Material	Molar percentage arabinose (assuming 100% total pentose)
Crude xylan,	6.2%
Xylan A (after 1 copper complex),	5.4%
Xylan E (after 6 copper complexes),	5.1%
Xylan F (final purified xylan),	see next paragraph.

In addition, Xylan F (23.41 mg.) was subjected to quantitative determinations using rhamnose hydrate (14.90 mg.) as a reference sugar (101), which was weighed in after hydrolysis and before neutralisation. The procedure followed for hydrolysis, neutralisation etc., was as before.

A correction was applied for the amount of sugar destroyed on hydrolysis under the conditions used. Pure, dry xylose (12.66 mg.) heated in a sealed tube with sulphuric acid (0.5 ml.; 1N; 95°) for 9 hours was estimated by the above method after the addition of rhamnose hydrate (14.88 mg.) and neutralisation. 11.17 mg. xylose was recovered indicating a loss under the conditions of hydrolysis of 11.7%. A correction was also applied for the fact that 0.88 g. xylan yields 1 g. xylose. Xylan F thus gave:-

	Detn.1.	Detn.2.
Xylose	88.2%	87.2%
Arabinose	0.5%	0.6%

Analysis of Xylan F.

(a) Lignin

The method of Mahood and Cable (165) was used. This involves treatment of the dried polysaccharide (0.4 g.) with sulphuric acid (72%: 5 ml.) at room temperature for 15 hours, followed by dilution to 3% boiling for 2 hours, and estimation of the washed, insoluble residue gravimetrically.

Lignin found, 1.54%

The method of Ritter (166) was also used. This involves treatment of the dry polysaccharide (0.4 g.) with sulphuric acid (72%: 3 ml.) at 20° for 2 hours, followed by dilution to 3%, boiling for 4 hours, and gravimetric estimation of the washed, insoluble residue.

Lignin found, 0.36%

(b) Methoxyl.

Volumetric methoxyl determination of Xylan F gave

OMe, 0.4%

(c) Ash, without addition of sulphuric acid, 0.43%

as sulphate, 0.74%

(d) Uronic Anhydride.

The method of Svenson, Mc.Ready and M'Lay (167) was used. In this method, the sample (150-200 mg.) is decarboxylated with 19% hydrochloric acid at 145° for 1½ - 2 hours, and the carbon dioxide liberated, after removal of hydrogen chloride, is absorbed in excess sodium hydroxide solution. The carbon dioxide is estimated by back titration of the sodium hydroxide solution against standard acid, after precipitation of

carbonates as the barium salt.

Under these conditions, an appreciable amount, (about 1%), of carbon dioxide may be given by simple sugars (177). A correction was therefore applied for the carbon dioxide liberated from pure xylose under the same conditions of boiling time, etc., as were used for the determination. No such blank value was available for the methylated polysaccharide sample.

Table 1.

Material	Boiling time.	CO_2 liberated.	Corrected CO_2	Uronic anhydride.
Xylose	90 mins.	0.62%	-	-
do.	120 mins.	1.10%	-	-
Xylan F.	120 mins.	2.20%	1.10%	4.4%
do.	90 mins.	1.36%	0.74%	3.0%
do.	90 mins.	1.45%	0.83%	3.3%
Methylated Xylan	90 mins.	1.19%	-	-

(e) Rotation, $[\alpha]_D^{20}$ - 93° (c, 0.21 in 4% sodium hydroxide).

Periodate oxidations.

Sodium metaperiodate was prepared from commercial sodium paraperiodate by recrystallisation from dilute nitric acid. The crystals obtained were washed repeatedly with distilled water until free from mineral acid.

The ethylene glycol used was neutral to methylene red-methylene blue indicator. On addition of excess ethylene glycol to the sodium metaperiodate solution used in the determination below, the resulting solution was also

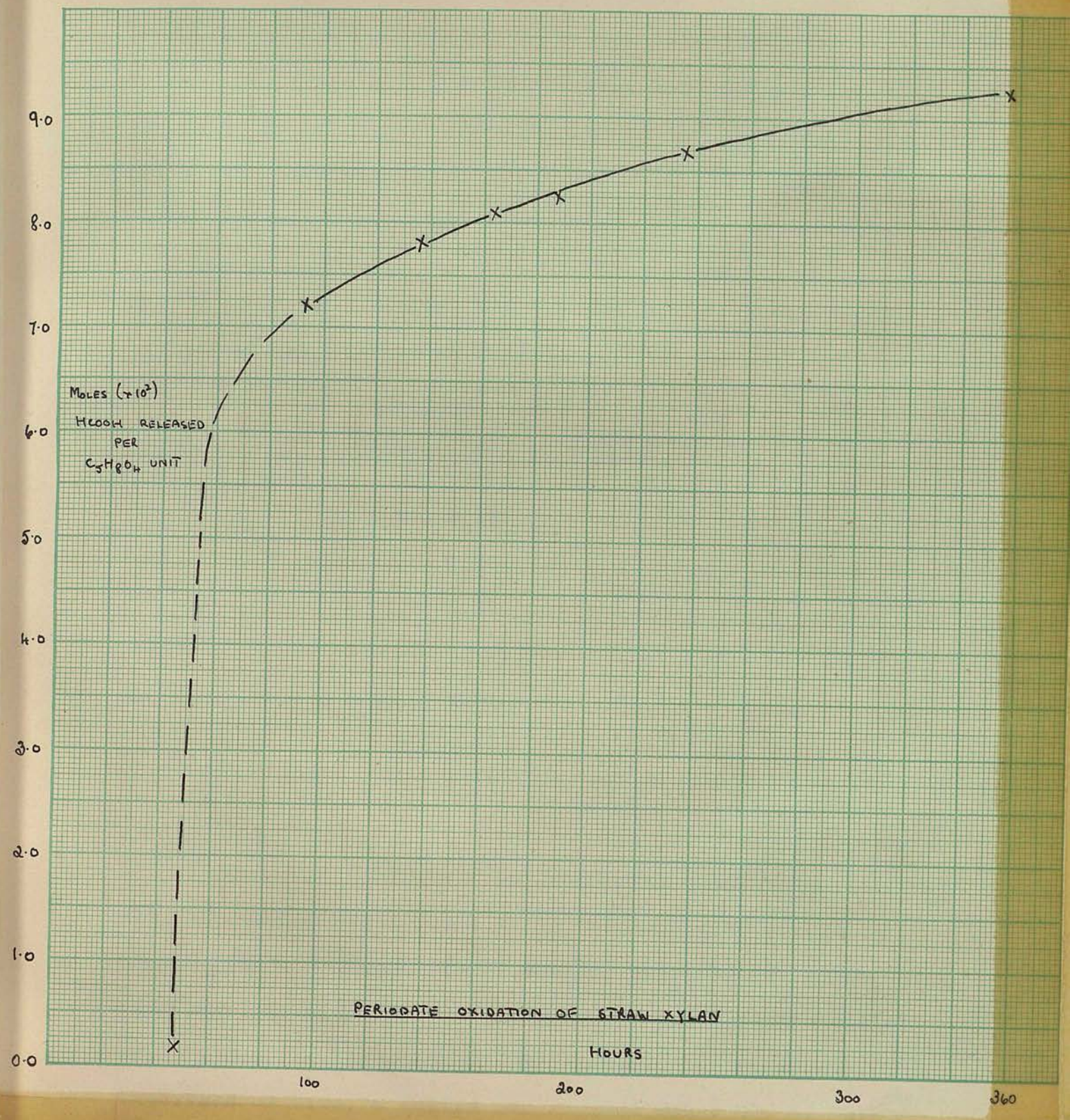
neutral to methyl red-methylene blue.

Estimation of Formic acid released.

The method of Halsall, Hirst, and Jones was used (11). The difficulty experienced by Chanda (140) consequent on the xylan forming an extremely fine suspension in the periodate solution, was met as follows:- Immediately prior to the withdrawal of a sample for the estimation of formic acid, the bottle was agitated and the contents thoroughly mixed. The calculations were then based on the assumption that a proportionate quantity of unoxidised xylan was removed with each sample. This assumption was felt to be justified in view of the extremely fine state of the suspension.

Two bottles were employed, one serving as a blank. Each contained potassium chloride solution (20 ml.;16%) sodium metaperiodate (40 ml.;0.1M), and water (40 ml.). One,in addition, contained the accurately weighed sample of dry xylan F., (350.7 mg.). The securely stoppered bottles were shaken continuously in the dark, removed at known intervals, agitated, and samples (10 ml.) of the suspension removed. Excess sodium metaperiodate was destroyed with neutral ethylene glycol (2 ml.), and, after standing for five minutes, the solution titrated against 0.0124N sodium hydroxide solution from a micro-burette, using methyl red-methylene blue indicator. Blank samples (10 ml.) were removed simultaneously and treated in a similar manner. The acidity of these was constant (0.140 ml.), and did not vary with time.

A sample of xylan F., (38.52 mg.) in distilled



PERIODATE OXIDATION OF STRAW XYLAN

water (2 ml.) was titrated against the standard sodium hydroxide solution, and was found to require 0.260 ml. of the alkali for neutrality to methyl red-methylene blue. The titre for each sample was, therefore, decreased by an amount corresponding to the acidity of the xylan contained in it, i.e., 0.236 ml. of 0.0124N sodium hydroxide solution.

The xylan F. used throughout this determination was, before use, steeped in water (30 mins.), decanted, and dried exhaustively at 40°/0.02 mm.

Table 2.

Hours.	10^2 x Moles formic acid/$C_5H_8O_4$ unit.
48	0.18
93	7.20
136	7.80
164	8.08
187	8.24
235	8.68
356	9.26

Periodate uptake.

A number of small bottles containing accurately weighed samples (40-50 mg.) of dry xylan F. and sodium metaperiodate solution (10 ml.;0.25M) were shaken in the dark together with bottles containing sodium metaperiodate solution. The latter served as blanks. After varying intervals of time, a bottle was removed and 5 ml. of the clear solution was extracted with a pipette. This was diluted to x 100 ml. in a graduated flask, and 10 ml. of

the diluted solution was used to find the periodate consumed (168). Excess sodium arsenite solution (25 ml.;0.0942N) was added, with potassium iodide (1 g.), and a little sodium bicarbonate. The mixture was left in the dark for 15 minutes and then titrated against standard iodine solution (0.1193N) from a micro-burette using starch as an indicator. The blank values did not vary with time.

Table 3.

Hours.	Moles periodate consumed/$C_5H_8O_4$ unit.
68	0.85
114	0.87
140	0.87
190	0.89
236	0.87

The remaining solution of oxidised polysaccharide was dialysed in a cellophane bag against tap water until free from inorganic ions, concentrated at 35°/18 mm. to small volume (5 ml.), hydrolysed with dilute sulphuric acid (2 ml.;2N;100°) for five hours and neutralised with barium carbonate. On examination of the liquid on a paper chromatogram against standard xylose, a trace of xylose was detected.

Reducing Power: 1. Hypoiodite oxidation.

To accurately weighed samples (110-160 mg.) of dry xylan F. which had been allowed to soak in water (5 ml.; 48 hrs.) was added iodine solution (10 ml.;0.1N) and phosphate buffer, pH 11.4 (5 ml.), (97). The solutions were contained in QQ boiling tubes, the stoppers of which

were moistened with potassium iodide solution (10%) to prevent loss of iodine. After standing in the dark, the solutions were acidified with sulphuric acid (2N) and the excess iodine was titrated against sodium thiosulphate solution (0.02306N).

Results obtained after 5 hours indicated that after this time oxidation was incomplete. After 16 hours, a value corresponding to one reducing group per 164 xylose residues was obtained.

2. Colorimetric method.

Meyer's method (169) using 3:5 - dinitrosalicylic acid in alkaline solution, was employed, as follows:-
A standard curve for xylose was first constructed. Two solutions of xylose were prepared containing 40.92 mg. and 10.13 mg. per 100 ml. respectively. By suitable dilution of these, a number of solutions containing known concentrations from 0.1 mg./5 ml. to 2.0 mg./5 ml. were obtained. To 5 ml. of each of these was added sodium hydroxide solution (1 ml.;6N) and 3:5-dinitrosalicylic acid solution (1 ml.;1.5%). The whole was then heated at 65° for 30 minutes, cooled and diluted to 25 ml. in a standard flask. Each solution was then compared with a similarly prepared blank in a Spekker photoelectric absorbtiometer using 1 cm. cells and green, 604 filters.

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Table 3A.

Standard Xylose.

Weight of xylose/ 25 ml.	10^6 x gm. mol./ 25 ml.	Mean value, log. $\frac{Io}{I}$
0.101 mg.	0.067	0.004
0.203 mg.	0.134	0.007
0.304 mg.	0.202	0.014
0.405 mg.	0.269	0.032
0.506 mg.	0.335	0.075
0.410 mg.	0.272	0.031
0.820 mg.	0.545	0.187
1.230 mg.	0.816	0.339
1.637 mg.	1.088	0.512
2.046 mg.	1.360	0.658

Dry xylan F (81.50 mg.) was dissolved in cold sodium hydroxide solution (1 ml.;6N), and water (5 ml.), and 3:5-dinitrosalicylic acid solution (1 ml.;1.5%) was added. The solution was heated, as described for the xylose standard solutions above, before being diluted to 25 ml.(a). Simultaneously three other solutions were prepared: (b) a similar weight of dry xylan F. was dissolved in cold sodium hydroxide solution (7 ml.;1N), and diluted to 25 ml. after heating at 65° for 30 mins; (c) sodium hydroxide solution (1 ml.;6N) was diluted to 25 ml. without heating, and (d) sodium hydroxide solution (1 ml.;6N) with 3:5-dinitrosalicylic acid solution (1 ml.; 1.5%) and water (6 ml.) were heated to 65° for 30 mins., and diluted, after cooling, to 25 ml. The total

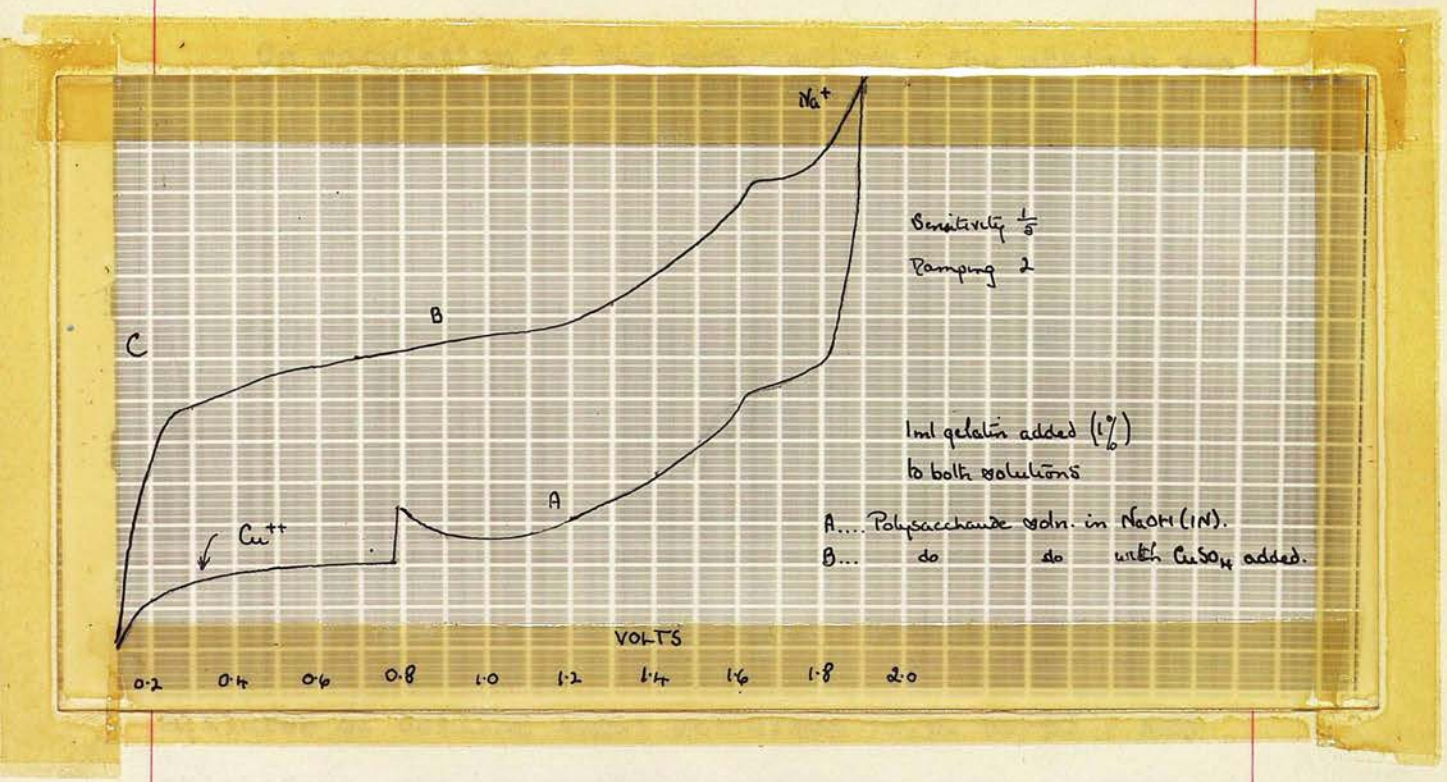
STANDARD CURVE.
Double point
mg Xylose / 25 ml.
0.00
0.10
0.20
0.30
0.40
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
1.0
1.1
2.1
3.1
4.1
5.1
6.1
7.1
8.1
9.1

absorbtion due to solutions (a), and (c) was compared with that due to the sum effect of (b), and (d), using four 1 ml. cells, the instrument and filters being as before. This procedure was necessary since solutions (b) and (d) cannot be mixed, reaction proceeding appreciably, in the cold, within the time taken to obtain readings. The reduced polysaccharide solution, on standing, became clouded within an hour.

Weight of Xylan F.	Mean $\log \frac{Io}{I}$	Xylose equivalent	Corrected Xylose equivalent.	D.P.
81.50 mg.	0.866	2.71 mg.	1.92 mg.	43

Polarogram.

It was attempted to reduce a solution of xylan F (50 mg.) in sodium hydroxide (25 ml.;1N) at a dropping mercury electrode using a Cambridge polarogram. Gelatin (1 ml.;1%) was added and nitrogen was passed through the solution for 5 minutes before reduction began. Readings were taken from 0.1 to 2.0 volts. The crest for Cu^{++} at 0.2 v., was distinguished by its reinforcement when copper sulphate solution was added to the solution in the cell. Crest(s), if any, due to the polysaccharide were indecisive.



Methylation of Xylan F.

Xylan F (18.4 g.) was suspended in water (150 ml.) in a 5 l. round-bottomed, three necked flask, and sodium hydroxide solution (150 ml.;4%) was added. The mixture was stirred for 12 hours in an atmosphere of nitrogen. To the clear solution, cooled in a water bath at 10°, was then added sodium hydroxide solution (150 ml.;50%), and dimethyl sulphate (180 ml.) was added dropwise over 8 hours, with vigorous mechanical stirring under nitrogen. A second methylation was performed using sodium hydroxide solution (300 ml.;40%) and with the dropwise addition of dimethyl sulphate (180 ml.) over 8 hours as before. After four such methylations, acetone (200 ml.) was added to aid solution of the partially methylated xylan, which separated out as a brown solid. Further quantities of acetone were added after the sixth (300 ml.), and the ninth methylations (300 ml.).

On completion of ten methylations, the acetone was removed at 25°/15 mm., the mixture cooled, neutralised (pH 8) by the careful addition of sulphuric acid (0.5N), heated to 60° and filtered hot through Buchner funnel and Whatman No.1 paper. The brown residue was washed repeatedly with hot water until the washings were free from sulphate, and dried, in air at room temperature, to a chocolate brown mass (20.04 g.).

Fractionation I.

The methylated xylan was fractionated using various mixtures of boiling light petroleum (b.p. 60-65°) and chloroform, the proportions used being given in Table 4. The light petroleum was purified before use by shaking with concentrated sulphuric acid (24 hours), neutralisation with sodium carbonate, drying over calcium chloride and redistillation. In each extraction, the solid was boiled gently under reflux for 1 hour with the specified solvent (150 ml.). The clear, supernatant liquid was separated hot by decantation. Each fraction was afforded 2 such treatments except for fractions No's.A5 and A6, which were given 3. Solvent from each fraction was removed at 25°/15 mm., and the residue was dried to constant weight. Fraction No. A1 was a yellow oil, later fractions being solids of increasing whiteness. Fraction No. A5 had $[\alpha]_D^{19}$ - 98.6° (c, 0.3 in chloroform).

Table 4.

Fraction.	Chloroform/ light petroleum.	Weight.	Yield.	Methoxyl.
A1	0:100	0.18 g.	0.9%	-
A2	10: 90	0.22 g.	1.1%	3.9%
A3	20: 80	0.18 g.	0.9%	15.7%
A4	25: 75	0.08 g.	0.4%	-
A5	30: 70	8.20 g.	41.0%	34.0%
A6	40: 60	10.59 g.	53.0%	32.9%
A7	Residue.	0.51 g.	2.3%	-

Further Methylation of Fraction No.A5.

This fraction (8.20 g.) was dissolved in neutral methyl iodide (135 ml.) using 10 ml. of dry methanol to aid solution. The mixture was heated on a water bath at 60°, under reflux, and silver oxide (100 g.) was added over a period of 24 hours. After cooling, the methyl iodide was removed by filtration, and the silver residue was extracted exhaustively with hot chloroform under reflux, the extract being added to the methyl iodide fraction. The solvent was then removed at 30°/15 mm.

The dry methylated xylan obtained was subjected to a second Purdie methylation using methyl iodide (135 ml.) as before, but without the addition of methanol. Silver oxide (70 g.) was used and heating at 60° under reflux was maintained for 24 hours. Extraction of the methylated xylan was achieved as before. The chloroform, methyl iodide extract was then combined with the extract obtained

after remethylation of Fractions No's.A6, and A7 (see below).

Further Methylation of Fractions A6, and A7.

These fractions were combined (11.10 g.), dissolved in acetone (250 ml.), and sodium hydroxide solution added (300 ml.;40%), followed by the dropwise addition of dimethyl sulphate (180 ml.) over 8 hours with vigorous stirring. The mixture was maintained in an atmosphere of nitrogen. The methylation was repeated a further three times after which acetone, sodium hydroxide and sodium sulphate were removed, successively as before. The crude, dry, methylated xylan, free from sulphate had methoxyl content 35.7%.

The partly methylated xylan was then dissolved in methyl iodide (135 ml.) and a little dry methanol (10 ml.), and treated with silver oxide (100 g.) in the manner used for methylation of Fraction No.A5. After extraction of the residues with hot chloroform, the solvent was removed at 25°/15 mm. The product (12.5 g.) had methoxyl content 38.0%.

Fractionation II.

The conditions employed and solvents used were the same as for Fractionation I. A yellow oil separated in Fractions No's. B1 and B2. The remaining fractions were white powders. There was marked swelling of the polysaccharide in solvent No.B3, and there was no residue from solvent No.B5. Two extractions (150 ml. each) were performed with each solvent except solvent No.B5, which had three.

Table 5.

Fraction.	Chloroform/ light petroleum.	Weight.	Yield.	Methoxyl.
B1	0:100	0.3 g.	3.4%	-
B2	10: 90			
B3	20: 80	1.2 g.	9.6%	37.9%
B4	25: 75	1.6 g.	12.8%	38.0%
B5	30: 70	9.4 g.	75.2%	38.2%

Fraction B5 had $[\alpha]_D^{19}$ - 82.7° (c, 0.45 in chloroform).

Viscosity.

The viscosity of methylated xylan (B5) was determined in m.cresol using an Ostwald viscometer. The solution (1.358%) was prepared in the cold, immediately before use. Mean time of flow for the solvent at 20 ± 0.1° was 251.2 seconds, and that for the solution, under the same conditions, was 359.5 seconds. In order to derive the molecular weight, the relation $\eta_{sp} = K_m Mc$ was used, the constant K_m being taken as 12×10^{-4}, which is the value derived for methylated cellulose in m.cresol (174). The remaining symbols refer to the molecular weight of the methylated xylan B5 (M); the concentration of xylan B5 in gram molecules of repeating unit per litre (c); and the specific viscosity (η_{sp}).

$$\eta_{sp} = \frac{T_2 - T_1}{T_1}$$

where T_2 and T_1 are the running times in seconds required by the solution and solvent respectively.

From the viscosity results, a value of 4,700 was obtained for the molecular weight of the methylated xylan B5, assuming the molecule to conform to the "ideal thread" conception, and that the above K_m value (12×10^{-4}) may properly be applied. This corresponds to a degree of polymerisation of 26-27 xylose residues. For a branched chain molecule, this will be lower than the correct value.

Hydrolysis of Methylated Xylan B5 and Estimation of Methylated Xyloses.

Methylated xylan Fraction B5 (50 mg.) was treated in a sealed tube with methanolic hydrogen chloride (5 ml.;1%) at 100° for 8 hours. After this time the acid was neutralised with silver carbonate, the mixture filtered, and the methanol removed at 25°/15 mm. The residual syrup was rehydrolysed with hydrochloric acid (5 ml.;0.5N) at 100° for 7 hours, the mixture cooled and neutralised with silver carbonate, filtered, and silver ions were removed by the passage of hydrogen sulphide and subsequent filtration. The clear solution was examined paper chromatographically in n - butanol; ethanol; water:ammonia (40:10:50:1) solvent, the developing spray being an aqueous solution of aniline oxalate (2%).

Sugars corresponding to trimethyl pentose (R_G 0.94), 2:3-dimethyl xylose (R_G 0.74), and monomethyl xylose (R_G 0.38) were identified in the hydrolysate against the relevant standards. A trace of uronic acid,

travelling close to the starting line, was also seen.

The molar ratios of the three methylated pentoses present were estimated using alkaline hypoiodite (96) and phosphate buffer, pH 11.4 (97), after separation of the mixture on quantitative paper chromatograms in n-butanol: ethanol:water:ammonia (40:10:50:1). In a single determination the trimethyl pentose strips from 3 papers were combined, (as were the monomethyl pentose strips), and these were estimated against the dimethyl xylose from a single paper. In each determination, the amounts of sugar mixture separated on each of the 3 papers was identical, the mixture being spotted on the chromatogram papers with a graduated capillary (cf. p.47). The filter strips containing the sugars were extracted with boiling water for 45 minutes by suspension below a reflux condensor. The sugar solutions were collected in QQ boiling tubes containing water (5 ml.). A blank was run for each sugar determined, the area of the blank being adjusted to equal the area of the paper strip extracted in the determination. Before extraction, the chromatograms were dried in air at room temperature, followed by 1 hour in an exhausted vacuum desiccator over water. The cooled sugar solutions were treated with iodine (1 ml.; 0.1N) from a micro-pipette followed by phosphate buffer (2 ml.). Double quantities of each reagent were used for the dimethyl samples, and for the dimethyl blank. Stoppers moistened with potassium iodide solution (10%) were placed in position and the tubes were stored in the dark (5-6 hrs.). After acidification with dilute sulphuric acid, the iodine present was estimated against 0.0075(8)N sodium thiosulphate using starch.

Table 6.

Sugar.	Detn.1. Molar percentage.	Detn.1. Wt.found by oxidation.	Detn.2. Molar percentage	Detn.2. Wt.found by oxidation.
2:3:4-trimethyl xylose	3.3%	0.74 mg.	2.8%	0.56 mg.
2:3-dimethyl xylose	93.5%	6.50 mg.	94.3%	5.80 mg.
2-methyl xylose	3.4%	0.65 mg.	2.9%	0.49 mg.

identified later.

Since the uronic acid present was not estimated, these results are expressed on the basis of percentage total pentose. (Cf. p.84 for total yields obtained by cellulose column separation of the hydrolysed, methylated xylan).

Hydrolysis of Methylated Xylan B5 and Separation by the Cellulose Column.

Fraction B5 (5.0 g.) was boiled gently under reflux with methanolic hydrogen chloride (300 ml.;0.5%). After 17 hours, some solid still remained undissolved. The acid strength was, therefore, increased to 0.84% and boiling under reflux continued a further 8 hours. The liquid at this stage was homogeneous to the centrifuge, yet translucent. Rotation values were therefore not obtained. The liquid was allowed to cool, and stand overnight but no solid separated.

The methanol was removed at 30°/15 mm. to a total volume of 75 ml., and the volume was sustained at 75-100 ml. by the continual addition, during distillation, of water (1 l.). The volume was then further reduced at 30°/15 mm.

to yield a clear, thin brown syrup (25 ml.). Hydrochloric acid (300 ml.;0.5N) was then added, upon which the mixture clouded and solid was deposited.

The mixture was boiled under reflux, and, after 5 hours the acid strength was increased to 0.7N. Boiling continued a further 5 hours. Since, after this time, complete solution had not been effected, the solid remaining was separated at the centrifuge, dried thoroughly at 30°/0.02 mm., and treated with methanolic hydrogen chloride (150 ml.;2½%) as before. Specific rotation became constant after 4 hours; +0.05°, +0.07° (1 hr.), +1.0° (4 hrs., constant). The methanol was removed as before, and the syrup obtained, together with the centrifugate above, was boiled under reflux with hydrochloric acid (300 ml.;0.5N) to constant rotation. $[\alpha]_{D}^{20}$ +31.7 ⟶ +34.5° (4 hrs., constant). It was observed, however, that on being allowed to stand, in the cold, the solution tended to become translucent.

The acid was neutralised on a well washed column (55 x 2 cm.) of Amberlite IR 4B(OH) ion exchange resin, and the water was removed at 40°/15 mm. At a total volume of 400 ml. the liquid was tested, and again found to be acid. The acid was therefore neutralised with calcium carbonate, the mixture filtered, and treated with batch ion exchange resins Amberlite IR 100(H) and IR 4B(OH). The mixture was then concentrated at 40°/15 mm. to a clear, light brown syrup and dried over phosphorus pentoxide and paraffin wax at 40°/0.02 mm. (4.9 g.).

Preparation of Cellulose Column.

Fine cellulose powder was obtained by grinding Whatman Ashless Filter Tablets, in a Wiley hammer mill, to pass 80 mesh. A column of this material was prepared in the usual way (170), and washed with water (2 l.), n-butanol (500 ml.), and 70:30 purified light petroleum (b.p.100-120°): butanol, saturated with water, (500 ml.)

Separation of Methylated Sugars on Cellulose.

The dry syrup (4.9 g.), obtained on hydrolysis of methylated xylan fraction B5, was dissolved in the minimum quantity of cold, purified butanol, and purified light petroleum (b.p.100-120°) was added until the ratio of these two solvents was approximately 30:70. The solution obtained was soaked into the top of the cellulose column (90 x 4 cms.), and allowed to dry for 2 hours. A constant head reservoir filled with light petroleum (b.p.100-120°):butanol mixture (70:30) saturated with water was then fitted into position, and the column was developed. All developing solvents were purified and distilled before use, the butanol being boiled under reflux (48 hours) over sodium hydroxide (40 g./l.), and the light petroleum shaken with concentrated sulphuric acid (200 ml./l.;12 hrs.) before re-distillation.

The eluate was collected in small tubes in an automatic fraction cutting device which changed the tubes every 6 minutes. After fraction 2 had been completely eluted, the speed of change was slowed to

one change in 12 minutes with replacement of the developing solvent by butanol saturated with water. Finally, (see Table 7), the aldobiuronic acid present on the column was removed with a water wash. Every tenth tube was evaporated to dryness, the contents taken up in a drop of water and sampled on a paper chromatogram in n-butanol:ethanol:water:ammonia (40:10:50:1). The relevant standards were run on each paper for comparison.

Fractions containing sugar of the same R_G value were bulked and the solvent removed at 40°/15 mm. The syrups obtained were dissolved in a little cold water, twice filtered through a well washed bed of "Filter-cel" and animal charcoal, to remove wax and colour, and again concentrated to syrups at 40°/15 mm. Final traces of water and impurity were removed by the addition of a little hot acetone (Analar) or dry methanol, filtration if necessary, and reduction to syrups at 35°/15 mm. Yields given in Table 7 for all fractions, except fraction 4, represent pure sugar.

Table 7.

Fraction.	Tube No.	Sugar.	R_G.	Weight.	Eluant at head of Column.
-	0-180	None.	-	-	Butanol:light pet.(30:70) saturated with water.
1	181-224	Trimethyl pentose.	0.94	0.149	do.
-	225-261	-	-	-	do.
2	262-782	2:3-dimethyl xylose.	0.74	4.180 g.	do.
-	783-1003	-	-	-	Butanol sat. water.
3	1003-1085	Monomethyl xylose.	0.38	0.139 g.	do.
-	1086-1150	xylose.	0.15	trace.	do.
4	1151-1359	Uronic acid.	(see text)	0.308 g.	water.

Examination of the Fractions.

Fraction 1.

The colourless syrup (149 mg.) did not crystallise on standing for several weeks at 0° C. Examination of the syrup on a paper chromatogram indicated a trace of monomethyl xylose to be present. This had probably been inadvertantly included during grouping of the tubes, and subsequent purification. The syrup was, therefore, separated on a thick paper (Whatman 3MM) with n-butanol: ethanol:water (4:1:5) solvent. The paper was washed with boiling water and dried before use, to remove waxes. The developed side strips contained standard sugars only, to allow the maximum of "end group" to be reclaimed.

The trimethyl xylose was recovered by extraction of the paper in a Soxhlet extractor with water. The solvent was removed at 35°/15 mm., and the resulting syrup was dissolved in a little cold water, and filtered free from wax over "Hyflo Supercel". The water was removed at 40°/15 mm., leaving a colourless syrup (144 mg.). The syrup had methoxyl content 47.6%, (Calc.for $C_8H_{16}O_5$, 48.4%). Purity by methoxyl was thus 98.3%, but alkaline hypoiodite oxidation in the presence of phosphate buffer, pH 11.4 (97) indicated 78.9% aldopentose. This suggested the presence of some dimethyl glycoside. A chromatogram developed in n-butanol:ethanol:water (4:1:5) and sprayed with aniline oxalate solution (saturated) containing syrupy phosphoric acid (2%), failed to confirm this due to excessive darkening of the paper on heating.

A sample (5.01 mg.) of syrup was hydrolysed in a sealed tube with sulphuric acid (2 ml.;0.5N) for 5 hrs. at 100°. The acid was neutralised with barium carbonate, and the resulting syrup on chromatographic analysis in n-butanol:ethanol:water (4:1:5) solvent showed some 2:3-dimethyl xylose (circ.20%) in addition to the trimethyl pentose.

The syrup remaining (133 mg.) was, therefore, hydrolysed with hydrochloric acid (20 ml.;3.65%) under reflux at 100° for 6 hours. $[\alpha]_D^{20}$ +23° (1 hr.) → 20° (5 hrs., constant). The acid was neutralised with silver carbonate, the mixture filtered and the filtrate with washings was treated with hydrogen sulphide to precipitate soluble silver. The silver sulphide was removed by filtration, the solution evaporated to 5 ml. at 40°/15 mm., refiltered, and finally evaporated to a syrup (130 mg.).

The syrup was separated on a semi-micro column of cellulose (50 x 1.5 cm.) which had been prepared, washed and tested in the usual manner (170). The eluant used was a mixture of n-butanol:light petroleum (b.p.100-120°), (30:70) saturated with water, the constituents of which mixture had been purified as before. A clear separation of the two components present was effected.

Table 8.

Fraction.	Tube No.	Sugar.	Weight.
-	0-70	-	-
1a	71-110	Trimethyl xylose	0.1011 g.
-	111-124	-	-
2a	125-138	2:3-dimethyl xylose.	0.0252 g.

The eluate was collected as before, every tenth tube being evaporated and sampled on a paper chromatogram. Fractions were bulked and the solvent removed at 35°/15 mm. The syrups obtained were dissolved in the minimum of cold water, filtered through charcoal and "filter cel", evaporated at 35°/15 mm., dissolved in a little acetone (Analar), refiltered, and finally evaporated at 40°/15 mm. to clear syrups.

Fraction 1a.

The purified syrup (101.1 mg.) deposited crystals immediately on removal of the acetone. They were straw brown, and had m.p. 87-8°. On re-crystallisation from dry ether, the colourless crystals obtained had m.p.89°, undepressed by admixture with authentic 2:3:4-trimethyl D xylose crystals. The crystals had OMe 48.2%, (Calc.for $C_8H_{16}O_5$, OMe 48.4%) and $[\alpha]_D^{14}$ +20° (c,0.9 in water). Alkaline hypoiodite oxidation, in the presence of phosphate buffer, pH 11.4, indicated 99.1% aldopentose.

Preparation of 2:3:4-trimethyl D xylose anilide.

(a) A sample of the crystalline fraction 1a (35.67 mg.) was boiled under reflux with freshly redistilled aniline (0.5 ml.) and dry ethanol (2 ml.) for 3 hours on a

boiling water bath. Solvent was then removed at reduced pressure leaving a syrup, which was set aside to crystallise at room temperature. After some weeks, large colourless plate crystals appeared. These were washed free from aniline, with ether, and had m.p. $>215^{\circ}$. The ether washings gave a pronounced smell of carbylamine. The crystals were set aside as being the product of a side reaction due to the heavy excess of aniline used, and the preparation was repeated (below).

(b) A sample of crystalline fraction 1a (24.1 mg.) was heated under reflux with freshly redistilled aniline (20 mg.) and dry ethanol (2 ml.) on a water bath, for 3 hours. After cooling, and removal of the solvent at reduced pressure, a syrup was obtained which crystallised on standing at 0° for a week. The crystals, recovered from adhering syrup and washed with dry ether had m.p. $96\text{-}98^{\circ}$ raised to 101° on recrystallisation from ether, undepressed by admixture with authentic 2:3:4-trimethyl D-xylose anilide.

Fraction 2a.

The semi-viscous syrup (25.2 mg.), purified as described above, had OMe 34.0%, (Calc.for $C_7H_{14}O_5$, OMe 34.8%). Purity, by buffered alkaline hypoiodite, was 98.1%. The syrup gave a discrete pink spot (R_G 0.74) on paper chromatographic examination in butanol:ethanol:water (4:1:5) and development with aniline oxalate solution (sat.).

Preparation of 2:3-dimethyl D xylose anilide.

Fraction 2a was entirely converted into the crystalline anilide using freshly distilled aniline (20 mg.) and dry ethanol. The mixture was heated gently for 3 hours under reflux on a water bath, cooled, and the solvent was removed at 15 mm. The solid which separated was washed free from aniline with dry ether yielding a cream-white powder. Recrystallisation from ethyl acetate-light petroleum gave colourless, needle crystals, m.p. 122° not depressed by admixture with authentic 2:3-dimethyl D xylose anilide recrystallised from the same solvent (see p.74), or by admixture with the anilide obtained from Fraction 2.

Examination of Fraction 2.

The viscous, straw coloured syrup (4.180 g.) after purification and prolonged drying at 30°/0.02 mm. had η_D^{20} 1.4694; OMe, 33.9% (Calc. for $C_7H_{14}O_5$, 34.8%), and $[\alpha]_D^{20}$ 22.9 , (c, 1.49 in water). Purity by buffered, alkaline hypoiodite was 98.4%. Paper chromatographic examination in n-butanol:ethanol:water (4:1:5) indicated a single discrete spot, R_G 0.74. The syrup did not crystallise spontaneously, even on long standing, but on seeding with a crystal of authentic 2:3-dimethyl D-xylose (178) the syrup yielded, at room temperature, large, colourless plates. Crystallisation took place extremely slowly over some months. The crystals, freed from adhering syrup, had, after prolonged drainage on a porous tile, m.p. 77-8°.

Preparation of anilide of Fraction 2.

A sample (26 mg.) of Fraction 2 was treated with freshly distilled aniline (20 mg.), and dry ethanol (2 ml.) in the same manner as for Fraction 2a. The anilide crystallised 12 hours after removal of the solvent at 15 mm. The solid was repeatedly washed with dry ether (x 5) to remove aniline, and a white powder was obtained which, after drying in air, had m.p. 134-5°. Mixed m.p. with an authentic sample of 2:3-dimethyl D-xylose anilide was undepressed.

Recrystallisation from ethyl acetate - light petroleum gave transparent needles, m.p. 123°. A mixture of these crystals with the above-mentioned sample obtained from ether had m.p. 123-30°. Nevertheless, on recrystallisation of the known sample from ethyl acetate - light petroleum, its m.p. was 122-3°, and a mixture of the two samples from the same solvent showed no depression.

Preparation of amide of Fraction 2.

A sample of Fraction 2,(100 mg.) was oxidised with bromine water (5 ml.) in an incubator at 37° until the mixture no longer reduced Fehling's solution. Excess bromine was removed by aeration, and the solution was neutralised with silver carbonate and filtered. Hydrogen sulphide was passed to remove soluble silver and the product was again filtered. Water was then removed at 40°/15 mm., and the resulting syrup was heated to 95° for 2 hours at 15 mm. to produce the lactone.

The dry, syrupy lactone was then treated with

saturated methanolic ammonia (2 ml.) at 0°, for 24 hours., after which time the solvent was removed at 35°/15 mm. to yield the crystalline amide. The crystals, recrystallised from ethyl acetate, had m.p. 132°, undepressed by admixture with authentic 2:3-dimethyl D-xylonamide.

Weerman reaction (184).

The crystalline 2:3-dimethyl D xylonamide was dissolved in water (1 ml.), sodium hypochlorite solution was added (0.5 ml.) and the mixture was kept for 3 hrs. at 0°. The excess of hypochlorite was destroyed with sodium thiosulphate and the solution was saturated with sodium acetate. A saturated solution of semicarbazide hydrochloride was then added but no precipitate was obtained, even on standing 12 hours.

A sample of gluconamide treated in the same manner yielded a white precipitate of hydrazodicarbonamide,

Examination of Fraction 3.

The syrup (139 mg.) crystallised on removal of water. A sample, recrystallised from methanol on the microscope slide, had m.p. 135-6°, mixed m.p. with a crystalline sample of authentic 2-methyl D xylose, 134-5°. Chromatographic examination of the crude crystals and adhering syrup in n-butanol:ethanol:water (4:1:5) showed a discrete spot R_G 0.39 (cherry red, u.v.), on a long paper. Using an analine oxalate spray, the colour of the spot differed markedly from that given by standard 3-methyl D xylose under the same conditions, the latter being brown-pink whilst standard 2-methyl D xylose, and Fraction 3 were purple-pink.

The crystals had OMe, 18.4% (Calc. for $C_6H_{12}O_5$, 18.9%); $[\alpha]_D^{19}$ + 30°, (c, 1.6 in water), and gave a purity by buffered alkaline hypoiodite of 99%.

Preparation of the Anilide of Fraction 3.

The anilide of fraction 3 was prepared in the manner described for previous fractions. The crystals, recrystallised from ethyl acetate had m.p. 123-4°, undepressed by admixture with authentic 2-methyl D xylose anilide crystals.

Purification and Examination of Fraction 4.

The crude fraction 4 (308 mg.) was a solid, incompletely soluble in ethanol, methanol or water. Paper chromatographic examination of a sample in n-butanol:ethanol:water (4:1:5) solvent showed two cherry red spots on development with saturated aniline oxalate solution. These had R_G values 0.09 - 0.10 (dipolar), and 0.05. In addition a yellow spot was detected, R_G 0.06, faintly visible in daylight but brightly in ultra violet. The fastest sugar, R_G 0.09 - 0.10 was heavily predominant.

(a) Demethylation. A sample (7 mg.) of crude fraction 4 was demethylated with hydrobromic acid (1 ml.; 48% w/w) at 100° in a sealed tube for 5 minutes, after which the acid solution was immediately diluted with water (10 ml.), and neutralised with silver carbonate (171). The mixture was filtered, and silver ions were removed with hydrogen sulphide. Examination of the product on a paper chromatogram against the relevant standards showed xylose, R_G 0.15; some 2-methyl

xylose, R_G 0.38; and a trace of 2:3-dimethyl xylose, R_G 0.74. No other sugars were detected.

(b) Hydrolysis.

A sample (10 mg.) of crude fraction 4 was hydrolysed in a sealed tube with sulphuric acid (2 ml.;1N) for 16 hours at 100°. There was a crystalline residue after this treatment which was removed by filtration, washed, and treated separately (see below). The hydrolysate was neutralised with silver carbonate, filtered, treated with hydrogen sulphide and refiltered. Examination of the product against standards on a paper chromatogram showed a trace of 2:3-dimethyl xylose, some 2-methyl xylose and two cherry-red spots, R_G 0.09-0.10 and R_G 0.16. The latter - which was probably 2:3:4-trimethyl glucuronic acid (?) - travelled just ahead of standard xylose. The irrigating solvent was n-butanol:ethanol:water (4:1:5).

A sample of the washed, crystalline residue obtained from the hydrolysis mixture was dried and treated with methanolic hydrogen chloride (2 ml.;5%) for 7 hours, at 100° in a sealed tube, neutralised with silver carbonate and filtered. Methanol was then removed at 35°/15 mm. The solid obtained was treated with sulphuric acid (2 ml.;1N) for 5 hours, neutralised with barium carbonate, and a clear solution was separated at the centrifuge. Examination of this solution on a paper chromatogram, and development with aniline oxalate solution showed reducing sugars to be absent.

The crystalline residue, examined under a polarising

microscope, was subsequently shown to be calcium sulphate, (monoclinic, oblique extinction).

(c) Reduction I (182,141.).

A sample (10 mg.) of crude fraction 4 was heated in a sealed tube with methanolic hydrogen chloride (2 ml.;1%) at a 100° for 6 hours. The solution was neutralised with silver carbonate, filtered, and evaporated at 40°/15 mm. to a clear syrup. This was dissolved in dry ether (20 ml.) and solid lithium aluminium hydride was added in small portions over 3 hrs. to a total of 100 mg. The mixture was heated to 30°, on a water bath, under reflux for this period. To the cooled solution, water was added cautiously to destroy the excess of lithium aluminium hydride. The mixture was then acidified with dilute sulphuric acid (2N), and the ether was removed at 30°/15 mm. The aqueous mixture remaining was concentrated to half volume (30°/15 mm.), and extracted repeatedly (x 4) with chloroform.

The chloroform extract was concentrated at 30°/15 mm. to a syrup and hydrolysed with hydrochloric acid (10 ml.; 0.5N) for 6 hours, neutralised with silver carbonate and filtered. Soluble silver ions were removed with hydrogen sulphide and subsequent filtration. Water was removed from the resulting solution at 30°/15 mm., and the product was examined on paper chromatograms in n-butanol:ethanol: water (4:1:5) solvent in comparison with the relevant standards. On development of the chromatograms with aniline oxalate solution (2%), sugars having the colours and R_G values of 2:3:4-trimethyl glucose (R_G 0.85, brown)

and 2-methyl xylose (R_G 0.38, pink) were identified in the mixture. Traces of a sugar corresponding to 2:3-dimethyl xylose (R_G 0.74;pink) were also present, and in addition, a yellow spot R_G 0.09. The proportions of 2:3:4-trimethyl glucose and 2-methyl xylose present were not equal, the latter on visual inspection appearing to be predominant.

(d) Reduction II.

The above reduction procedure was repeated with a fresh sample (11 mg.) of crude fraction 4 which was treated as before, with the exception that the reduction mixture was neutralised (not acidified) with dilute sulphuric acid (2N) after the destruction of the excess of lithium aluminium hydride with water. Ether was removed at 20°/15 mm., and the resulting aqueous mixture was not further reduced in volume. Chromatographic examination of the final, hydrolysed product showed the same sugars to be present, in approximately the same proportions as before, with the exception that 2:3:4-trimethyl glucose now equalled the 2-methyl xylose in quantity.

The disparity between the results of Reductions I and II was later found to be due, not to the difference in procedure, but to the fact that chromatograms on the product of Reduction I had been roasted for too short a time after spraying with aniline oxalate solution. There is a significant difference between the times taken for the two sugar spots to develop; that given by 2:3:4-trimethyl glucose needing the longer time. On subsequently re-roasting old chromatograms on the Reduction I product,

the spots of 2:3:4-trimethyl glucose could be developed to their full strength, and then these appeared in 1:1 ratio with those of 2-methyl xylose.

Purification of Fraction 4.

The remaining crude Fraction 4 (270 mg.) was dissolved in the minimum quantity of hot methanol, cooled and filtered. Samples of the clear solution were oxidised on paper chromatograms, as described below, with sodium metaperiodate. The bulk of the solution was evaporated to dryness at 30°/15 mm., and rehydrolysed with hydrochloric acid (20 ml.;1N) for 5 hours. The acid was neutralised with silver carbonate, and the mixture was filtered, treated with hydrogen sulphide to remove soluble silver, and refiltered. Removal of water at 40°/15 mm. yielded a semi-solid brown syrup (101 mg.).

Periodate Oxidation on Paper Chromatograms (172).

A chromatogram was prepared having two spots of the methanolic solution of crude fraction 4 at intervals on the starting line, and this was eluted with n-butanol: ethanol:water :ammonia (40:10:50:1) solvent. The dry paper was bisected longitudinally and one half was developed in the normal manner with saturated aniline oxalate solution. The second half was sprayed with a solution of sodium metaperiodate (5%), and oxidation was allowed to proceed in the dark, at room temperature, for 5 minutes. After brief drying at 60°, the paper was exposed to sulphur dioxide in a desiccator, sprayed lightly with neutral ethylene glycol and allowed to stand for a further 5 minutes. The paper was again dried at 60°,

exposed to sulphur dioxide for a short time (2-3 mins.), and a very thin spray of freshly prepared Schiff's reagent was then applied. The paper was finally developed at 60° in the air oven. To obtain a light background it is essential that the Schiff's reagent be applied very sparingly, and that the temperature of the developing oven is not too high. On standing in light, the background quickly becomes coloured.

The spot corresponding to the uronic acid having R_G 0.09-0.10 showed the magenta spot of a Schiff-aldehyde complex after this treatment. The sugar having R_G 0.05 gave no reaction with the Schiff's reagent.

A chromatogram was prepared having two spots of the methanolic solution of crude fraction 4 at the starting line, together with duplicate spots of a standard glucose solution. The spotting was arranged so that, on subsequent bisection of the paper, spots of fraction 4 and of glucose were present in each half. The chromatogram was eluted, dried, and bisected in a similar manner as before, half being treated with saturated aniline oxalate solution and the other half treated with sodium metaperiodate solution (5%). After 7 minutes, a spray of ethylene glycol was applied to the dry paper, and 5 minutes were allowed in the dark, at room temperature, for the destruction of excess of periodate. A spray of freshly prepared potassium iodide solution (5%) was then applied, and the position of the glucose standard was indicated by an iodine stain. No iodine was detected from the sugars present in fraction 4 having R_G values

of 0.09-0.10 and 0.05, indicating that no formic acid had been released by these sugars on treatment with periodate.

Purification of Fraction 4.

The hydrolysed fraction 4 (101 mg.) was separated on wide strips of filter sheet (Whatman 3MM) which had previously been eluted with hot water and with the solvent used, and dried. The eluting solvent used was n-butanol: ethanol:water:ammonia (40:10:50:1). Side strips, which were removed and developed to show the position of the sugars, were spotted with standard 2:3-dimethyl D xylose (R_G 0.74). Preliminary paper chromatography had indicated that, apart from a uronic acid of low R_G, this was the only sugar present in the hydrolysis mixture.

The strips of filter sheet bearing the different sugars were extracted in a Soxhlet extractor with methanol: water (50:50 v/v), yielding two fractions:-

Fraction (a).

This was evaporated at 35°/15 mm. to a syrup, dissolved in the minimum of cold water, filtered through a well washed bed of "Filter-cel" and charcoal, and evaporated to dryness at 35°/15 mm. The syrup, examined on a paper chromatogram in n-butanol:ethanol:water (4:1:5) solvent, gave a single, discrete pink spot, R_G 0.74 corresponding to 2:3-dimethyl xylose. The amount of 2:3-dimethyl xylose present, estimated by rotation, was 20 mg.

Fraction (b)

This was evaporated at 35°/15 mm. to a syrup, dissolved in the minimum of methanol, filtered, and the methanol was removed at 35°/15 mm. The syrup was then dissolved in

water, filtered, and the solution was finally evaporated at 35°/15 mm. to yield a semi-solid syrup (72 mg.). Fraction (b) had, $[\alpha]_D^{16}$ +97.5° (c,0.72 in water); OMe, 32.2%; equiv., 350. (Calc. for $C_{15}H_{26}O_{11}$, OMe, 32.4%; equiv., 382).

Reduction of Fraction 4(b).

This fraction (13 mg.) was treated with methanolic hydrogen chloride (2 ml.;1%) for 5-6 hours, and neutralised in the usual way. The syrupy ester glycoside resulting was dissolved in dry ether and reduced with lithium aluminium hydride as described previously for the crude fraction 4. The reduction product was extracted exhaustively with chloroform, and hydrolysed with hydrochloric acid (10 ml.,0.5N) for 6 hours. Neutralisation of the solution was accomplished with silver carbonate. The mixture was then filtered and the silver carbonate residue was thoroughly washed with water, the washings being added to the filtrate. Soluble silver was removed with hydrogen sulphide, and the mixture was again filtered and washed.

The mixture of 2-methyl xylose and 2:3:4-trimethyl glucose obtained was separated on a filter sheet (Whatman No.1) eluted with n-butanol:ethanol:water (4:1:5) solvent. Side strips bearing standard sugars were used. The centre strips were extracted for 45 minutes with boiling water, whilst suspended on hooks from spiral, reflux condensors. The sugar solutions were collected in B24 QQ boiling tubes, and were estimated with alkaline hypoiodite (96), using iodine solution (1 ml.,0.1N) and phosphate buffer, pH 11.4 (2 ml.), (97).

Back titration of excess iodine was performed against sodium thiosulphate (0.00758N) using a micro-burette. Titre figures, 2:3:4-trimethyl glucose: 2-methyl xylose; 0.377 ml.: 0.412 ml. These results indicate a proportionality between these two sugars of 1.0:1.1 mol.

Fraction 4(b); other experiments.

The equivalent of the pure uronic acid was determined by direct titration of a solution of the acid against carbonate-free sodium hydroxide (0.01002N) using phenolphthalein. (see p. 83).

A portion of the solution of the sodium salt so obtained (53.6 mg. calculated as free acid), was then treated with sodium meta-periodate (3 ml.;0.25N) in the dark, and periodate consumed was estimated using sodium arsenite solution (0.1N) and standard iodine (0.1070N) as described previously. After 26 hours an uptake of 0.8 mol. periodate per $C_{15}H_{26}O_{11}$ unit was obtained.

Separation Summary.

Cellulose column separation of the hydrolysed, methylated xylan B5 thus yielded the weights of sugars given in Table 9. The weights quoted are those calculated to be in the original hydrolysis mixture, by correcting for losses during separation.

Table 9.

Sugar.	Wt. present in hydrolysis mixture.	Mol.%.
2:3:4-trimethyl D xylose.	120 mg.	2.30
2:3-dimethyl D xylose.	4.522 g.	93.46
2-methyl D xylose.	148 mg.	3.32
Tetramethyl aldobiuronic acid.	97 mg.	0.93

Hydrolysis of Xylan F and examination of the Acidic Fraction.

Note: In the following section "oligosaccharide-uronic acid" is taken to mean a uronic acid residue linked to a disaccharide or oligosaccharide residue; and in the present instance the disaccharide or oligosaccharide residue referred to is comprised of xylose units only.

A quantity (20-25 g.) of xylan F was hydrolysed under reflux with sulphuric acid (400 ml.;1N) at 100° for 6-7 hours, and the hydrolysate was neutralised on a column of Amberlite ion exchange resin IR 4B(OH). The neutral eluate (A) was collected, evaporated to a syrup at 35°/15 mm., and set aside. The resin was then treated with sulphuric acid (2N) until the eluate was permanently acid, and with distilled water until the eluate became free from SO_4^{--}. The acid and aqueous eluates were collected, combined, and partially neutralised with barium hydroxide (2N). The volume was then reduced to 300 ml. at 35°/15 mm., and the mixture was neutralised with barium carbonate, allowed to stand for 48 hours, filtered and concentrated to a neutral syrup (B) at 35°/15 mm.

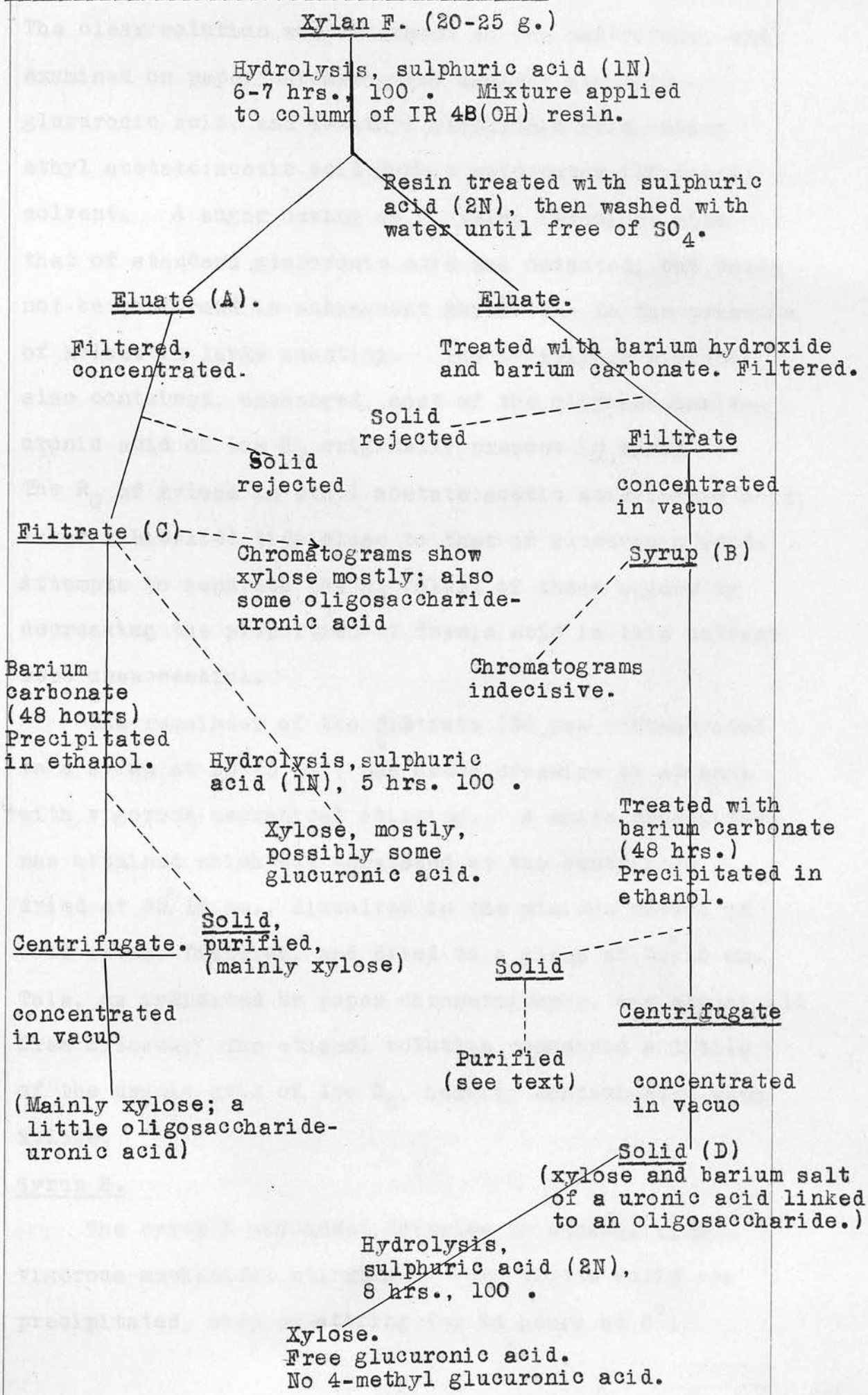
Paper chromatographic examination of syrups A, and B, in n-butanol:ethanol:water (4:1:5); n-butanol:ethanol:water:ammonia (40:10:50:1); and ethyl acetate:acetic acid:formic acid:water (18:3:1:4) solvents showed that A was mainly free xylose but, in addition, contained some of the uronic acid, possibly as an oligosaccharide-uronic acid unit which had not been removed by the IR 4B(OH) resin.

Syrup B was found to contain a little uronic acid together with a quantity of free sugar which had been adsorbed with the acid on the resin. The complete separation of xylose and uronic acid using IR 4B(OH) resin was thus found to be ineffective. This may have been due to the fact that most, if not all, of the uronic acid was present as aldobiuronic acid and possibly oligosaccharide-uronic acid units; i.e., as molecules which were too large to be effectively adsorbed by the resin. In support of this it was found that the R_G value of the uronic acid present in both fractions was extremely low in ethyl acetate:acetic acid:formic acid:water (18:3:1:4), the uronic acid travelling half the distance travelled by glucuronic acid. In n-butanol:ethanol:water (4:1:5) solvent, the uronic acid remained unmoved at the starting line when glucuronic acid had travelled 6 cms., and glucurone had travelled 23.5 cms.

Syrup A.

Water (40 ml.) was added to syrup A, and the solution was treated with excess of barium carbonate at room temperature for 48 hours, and filtered (C). Paper chromatographic examination of the filtrate in ethyl acetate:acetic acid:formic acid:water (18:3:1:4), and n-butanol:ethanol:water:ammonia (40:10:50:1) solvents, in comparison with standard barium 4-methyl glucuronate, and barium glucuronate was indecisive. A sample of the filtrate (C) was hydrolysed with sulphuric acid (2 ml.;1N) for 5 hours at 100°, and neutralised with barium carbonate.

Summary: Separation of Acidic Fraction.



The clear solution was separated at the centrifuge, and examined on paper chromatograms against standard glucuronic acid, and 4-methyl glucuronic acid, using ethyl acetate:acetic acid:formic acid:water (18:3:1:4) solvent. A sugar having an R_G value identical with that of standard glucuronic acid was detected, but could not be confirmed in subsequent papers due to the presence of xylose in large quantity. The hydrolysis mixture also contained, unchanged, most of the oligosaccharide-uronic acid of low R_G originally present in syrup A. The R_G of xylose in ethyl acetate:acetic acid:formic acid: water (18:3:1:4) lies close to that of glucuronic acid. Attempts to separate the R_G values of these sugars by decreasing the proportion of formic acid in this solvent were unsuccessful.

The remainder of the filtrate (C) was concentrated to a syrup at 35°/15 mm., and added dropwise to ethanol with vigorous mechanical stirring. A white precipitate was obtained which was separated at the centrifuge, dried at 35°/15 mm., dissolved in the minimum amount of cold water, filtered, and dried to a glass at 35°/15 mm. This, as indicated by paper chromatography, was almost all free xylose. The ethanol solution contained a little of the uronic acid of low R_G, heavily contaminated with xylose.

Syrup B.

The syrup B was added dropwise to ethanol with vigorous mechanical stirring. Very little solid was precipitated, even on storing for 24 hours at 0°.

The small amount of solid obtained was separated at the centrifuge, dried at 35°/15 mm., redissolved in the minimum quantity of cold water, and finally evaporated to dryness at 35°/15 mm. Paper chromatographic examination showed the solid to contain approximately equal quantities of xylose and oligosaccharide-uronic acid.

The supernatant alcoholic solution was evaporated to dryness at 40°/15 mm. to yield a yellow-white solid (D), which was completely water soluble. An aqueous solution of this solid gave a white precipitate with dilute sulphuric acid (2N), indicating that a soluble barium salt was present. Paper chromatographic examination of this solid (D) as the barium salt in n-butanol: ethanol:water (4:1:5) solvent showed the oligosaccharide-uronic acid of low R_G to be present, together with a little xylose. Confirmation of this was obtained by examination of the solid (D) in ethyl acetate:acetic acid: formic acid:water (18:3:1:4) solvent against standard glucurone, glucuronic acid and 4-methyl glucuronic acid. The uronic acid present in solid (D) was of much smaller R_G than any of the above standards.

The solid (D) was therefore hydrolysed with sulphuric acid (2 ml.;2N) for 8 hours at 100°, and neutralised with barium carbonate. The supernatant liquid, separated at the centrifuge, was examined paper chromatographically in n-butanol:ethanol:water:ammonia (40:10:50:1), n-butanol: ethanol:water (4:1:5), and ethyl acetate:acetic acid: formic acid:water (18:3:1:4) solvents against standard glucurone, glucuronic acid, and 4-methyl glucuronic acid.

On development with saturated aniline oxalate solution, sugars corresponding both in colour and R_G value to glucuronic acid and glucurone were observed. No 4-methyl glucuronic acid was present. In the formic acid solvent, in addition to some xylose and glucuronic acid, a faint brown spot having an R_G value approximately half that of xylose was detected. This moved 10.1 cms. when the distances travelled by xylose, 4-methyl glucuronic acid and glucuronic acid were 25.7, 22.6 and 18.2 cms., respectively.

Acetylation of Xylan F.

Xylan F (600 mg.) was dispersed in formamide (60 ml.) at 50° by vigorous stirring for 1½ hours, (173). Purified, dry pyridine (56 ml.) was then added, followed by the dropwise addition of acetic anhydride (26 ml.) over 1 hour, at 45°. Vigorous mechanical stirring was sustained for this time. The mixture was then shaken overnight in the dark at room temperature. The acetate was precipitated by the addition of the clear, dark solution to ice cold water (2½ l.) under vigorous stirring. The precipitate was washed free from pyridine by repeated agitation in water and removal of the wash liquid at the centrifuge. It was then washed several times in alcohol and dried in air to a white powder.

The solid was dispersed in dry pyridine (56 ml.) at 70° with brisk mechanical stirring, and acetic anhydride (30 ml.) was added over 4 hours at 45-50°. The mixture was shaken in the dark overnight, and the acetate was regenerated as before. Yield, 1.0 g.

Acetyl determination.

The acetyl content was determined in the usual way by saponification of the acetate with excess sodium hydroxide in aqueous acetone (50:50, analar.) and, after standing for 20 hours at room temperature, back titration to a phenolphthalein end point with standard hydrochloric acid (0.1N). Found, acetyl 39.6%. (Calc. for xylan diacetate, 39.8%).

Determination of Molecular Weight by Isothermal Distillation (216).

The M.W. of methylated wheat straw xylan (fraction B5) was determined by isothermal distillation of a solution in benzene (0.0929 g. in 20 ml.) at 22.5±0.01° by courtesy of Dr. C.T. Greenwood. A value of 8,000 500 was obtained for the molecular weight, corresponding to a D.P. of 47-53 xylose residues.

EXPERIMENTAL.

PART II

HEMICELLULOSE A FROM BEECHWOOD.

The beechwood hemicellulose A used in the following investigations was kindly made available by Mr. I.R.C. MacDonald of the Forest Products Research Laboratory, Princes Risborough. It was isolated from European beechwood (Fagus sylvatica), by extraction of the wood with sodium hydroxide (1.0N) (179). The wood was not delignified chemically before extraction. The polysaccharide was received as a fine white powder and for the analyses in Table 1 the Forest Products Research Laboratory is responsible.

Table 1.

Methoxyl,	4.5%
Pentosan, calculated as xylose, estimated by furfural and uncorrected for uronic anhydride,	84.8%
Pentosan, corrected for uronic anhydride,	81.4%
Uronic anhydride,	10.4%
Sulphuric acid lignin,	0.9%
Acid soluble lignin,	1.0%
Total polysaccharide,	91.1%
Ash, as Na_2O,	1.56%
(c, 0.4715 in 1N sodium hydroxide), $[\alpha]_D$	- 90.6°

Unless specifically stated, experiments described in the following pages were performed in the same manner as for straw xylan on the pages indicated.

It was decided to repeat, and confirm the above estimates of uronic anhydride, methoxyl, total pentose, and rotation for beechwood hemicellulose A.

Methoxyl.

Determined by the volumetric method of Viebock and

Brecher (197,198,199.) the following results were obtained:-

Table 2.

Weight taken:	17.37 mg.	15.35 mg.	5.62 mg.
Methoxyl:	1.71%	1.65%	2.0%

The experimental error is here increased due to the extremely small titration figure involved.

By the gravimetric method of Zeisel (199) a value of 4.9% methoxyl was obtained. This figure is in agreement with the value of 4.5% obtained at the Forest Products Research Laboratory, where the methoxyl content was also obtained gravimetrically. However, not all the precipitate which was weighed as silver iodide was, in fact, silver iodide. This was obvious from its appearance and dark brown colour.

Uronic anhydride (cf. p.50.)

Table 3.

Boiling Time.	CO_2 liberated%.	Xylose blank CO_2%.	Corrected CO_2%.	Uronic anhydride %.
2 hours.	3.99	1.10	2.89	11.5
1½ hours.	2.95	0.62	2.33	9.3
1½ hours.	3.02	0.62	2.40	9.6

Rotation. $[\alpha]_{\lambda}^{20}$ -89.4°, (c, 0.347 in 4% sodium hydroxide.).

Quantitative determinations of sugars present.

1. Preliminary hydrolysis.

A sample (10-15 mg.) of hemicellulose A was hydrolysed with sulphuric acid (2 ml.;1N) for 10 hours at 100°, neutralised with barium carbonate, and the neutral hydrolysate was examined on paper chromatograms

in n-butanol:ethanol:water (4:1:5) and n-butanol:benzene: pyridine:water (5:1:3:3) solvents. Some uronic acid of low R_G, having the characteristic heart shaped spot was detected, together with xylose and a trace of rhamnose. No other sugars were present.

2. Estimation of xylose (cf.p.49).

A sample (58.18 mg.) of polysaccharide was hydrolysed with sulphuric acid (2 ml.;1N) for 9 hours at 100°. After neutralisation, and separation of the mixture on paper chromatograms, xylose was estimated using sodium metaperiodate (175). Galactose (57.58 mg.) was used as a reference sugar (101). The areas of paper eluted in each single determination were adjusted, by blanks, to be the same for each sugar. The duration of the oxidation was 20 minutes, on the boiling water bath.

	Detn. 1.	Detn. 2.
Xylose	81.4%	81.8%

These figures are corrected for loss on hydrolysis.

3. Estimation of rhamnose.

A sample (58.52 mg.) of polysaccharide was hydrolysed, and the neutral hydrolysate was separated on a number (8) of paper chromatograms in the usual way. Sugars were extracted from the paper by cold water elution (164) and estimated by the method of Nelson (180,185.). Standard galactose (53.44 mg.) was used as a reference sugar. The total galactose present in the 8 papers was eluted, made up to 100 ml. with distilled water, and samples (2 ml.) were taken for the determination. Standard solutions containing 0.1 to 0.4 mg. of rhamnose, and of

galactose, and also water and paper blanks, were treated simultaneously.

The solutions were heated at 100° for 15 minutes to equalise the amount of oxygen in each. After cooling, the copper reagent (2 ml.;see below) was added to each, and the solutions were again heated at 100° for 20 minutes and cooled. Arsenomolybdate reagent (2 ml.) was then added, followed by water sufficient to make each sample up to 25 ml. by weight. The sugars present were then estimated colorimetrically in a Spekker photoelectric absorbtiometer using green, 604 filters. The standard solutions were measured against the water blanks in the Spekker, and a graph was prepared for each sugar. From these graphs the weights of sugar in the unknown samples could be obtained from their absorbtion values.

Copper reagent.

This was obtained by mixing copper solutions A, and B, below in the ratio 25:1 on the day of use.

Copper solution A.

Anhydrous sodium carbonate (25 g.), sodium hydrogen carbonate (20 g.), anhydrous sodium sulphate (200 g.), and sodium potassium tartrate (25 g.) made up to 1 litre of solution with water. Sediment, if any, is removed by filtration and solution is stored at 20°.

Copper solution B.

Copper sulphate solution (15%) containing 1 or 2 drops concentrated sulphuric acid per 100 ml.

Arsenomolybdate reagent.

Ammonium molybdate (25 g.) is dissolved in water

(450 ml.) and concentrated sulphuric acid (21 ml.) is added. A solution of sodium hydrogen arsenate septahydrate (3 g.) in water (25 ml.) is then added, the mixture mixed thoroughly, and incubated at 37° for 24 hrs. The solution is best stored in a brown bottle.

Table 4.

	Volume.	Wt. of sugar.	Mean reading	log $\frac{I_o}{I}$
Standard rhamnose hydrate solution.	1 ml.	0.1122 mg.	0.05	
	2 ml.	0.2244 mg.	0.10	
	3 ml.	0.3366 mg.	0.15	
	5 ml.	0.5610 mg.	0.25	
Standard galactose solution.	1 ml.	0.1182 mg.	0.08	
	2 ml.	0.2364 mg.	0.16	
	3 ml.	0.3546 mg.	0.26	
	5 ml.	0.5910 mg.	0.43	
Galactose	2 ml.	0.4100 mg.	0.30	
Rhamnose (hydrate)	-	0.090 mg.	0.04	

This corresponds to a value for rhamnose of 0.40%, calculated as rhamnose hydrate.

Purification.

Removal of the rhamnose present in beech hemicellulose A was attempted by various means.

1. Copper complex formation.

A sample (2 g.) of polysaccharide was dissolved in sodium hydroxide (100 ml.;4N) and the copper complex was formed by the addition to it, of Fehling's solution. The complex was filtered free from solution, washed, and the polysaccharide was regenerated in ethanol as described for straw xylan. Paper chromatographic examination

of the product showed rhamnose to be present in the same proportion as before complex treatment. A sample of polysaccharide which was afforded two such complex treatments, when regenerated and hydrolysed showed rhamnose to be present in the hydrolysate still in unchanged amount.

2. Fractional solution.

A sample (circ. 3 g.) of dry polysaccharide was boiled under reflux with aqueous acetone (50%;v/v;160 ml.) for 4 hours. The mixture was separated hot at the centrifuge, washed with aqueous acetone followed by acetone, and then ether and dried at 15 mm. Paper chromatography of a sample hydrolysate (sulphuric acid; 1N; 6 hrs.; 90°) showed the rhamnose content to be unchanged.

3. Fractional precipitation.

(a) Small scale.

A sample (1.5 g.) of polysaccharide from the above treatment was dissolved in sodium hydroxide (5 ml.;4%) and glacial acetic acid was added until a precipitate just appeared, (pH 7). A colloidal solution was separated from a solid precipitate (I) at the centrifuge. The colloidal solution gave no further precipitate on addition of more glacial acetic acid to acidity. The solid (I) was washed with water, ethanol, and ether at the centrifuge, and dried in air to a white powder. To the acid, colloidal solution was added a drop of dilute hydrochloric acid (2N), followed by ethanol (1 vol.), and a gelatinous mass (II) was precipitated. Further

addition of ethanol did not increase the quantity of the precipitate. Solid (II) was washed at the centrifuge with water, ethanol, and ether, and dried in air to a white powder.

Samples of the hydrolysates of I and II were compared on paper chromatograms with a sample hydrolysate of untreated polysaccharide. It was found that rhamnose had been removed from sample II but, in addition, no uronic acid appeared to be present in this sample, even on very heavy spotting.

(b) Large scale.

The above small scale separation was repeated with 30 g. of hemicellulose. Yields of fractions obtained were :- I, 23.4 g.; II, 4.0 g. Examination of the hydrolysates of fractions I and II on paper chromatograms in n-butanol:ethanol:water (4:1:5) solvent appeared to confirm the results of the small scale fractionation. The hydrolysate of fraction II gave a white patch, undeveloped by saturated aniline oxalate solution covering the position near the starting line where a brown spot of uronic acid was visible in the hydrolysate of fraction I. However, a uronic anhydride determination (167), gave uronic anhydride, 9.8%.

4. Other methods.

To a sample (0.1 g.) of fraction I was added cold sodium hydroxide (0.01N;3 ml.), and the mixture was allowed to stand for 24 hours. The supernatant liquid was decanted carefully, and further cold sodium hydroxide (0.01;3 ml.) was added, the mixture being again left

to stand (24 hrs.). The combined centrifugates were then acidified with hydrochloric acid (2N), ethanol (1 vol.) was added, and the precipitate so obtained was separated at the centrifuge, washed with water, ethanol, and ether and air dried. The solid residue remaining from the alkaline extractions was similarly washed and dried. Paper chromatographic examination of sample hydrolysates of both fractions showed that no effective separation had been accomplished.

On preparing a suspension in water of a sample of untreated hemicellulose A and examination paper chromatographically, no rhamnose could be detected, indicating that this sugar was not present in the free state.

Periodate Oxidation.

1. Periodate uptake.

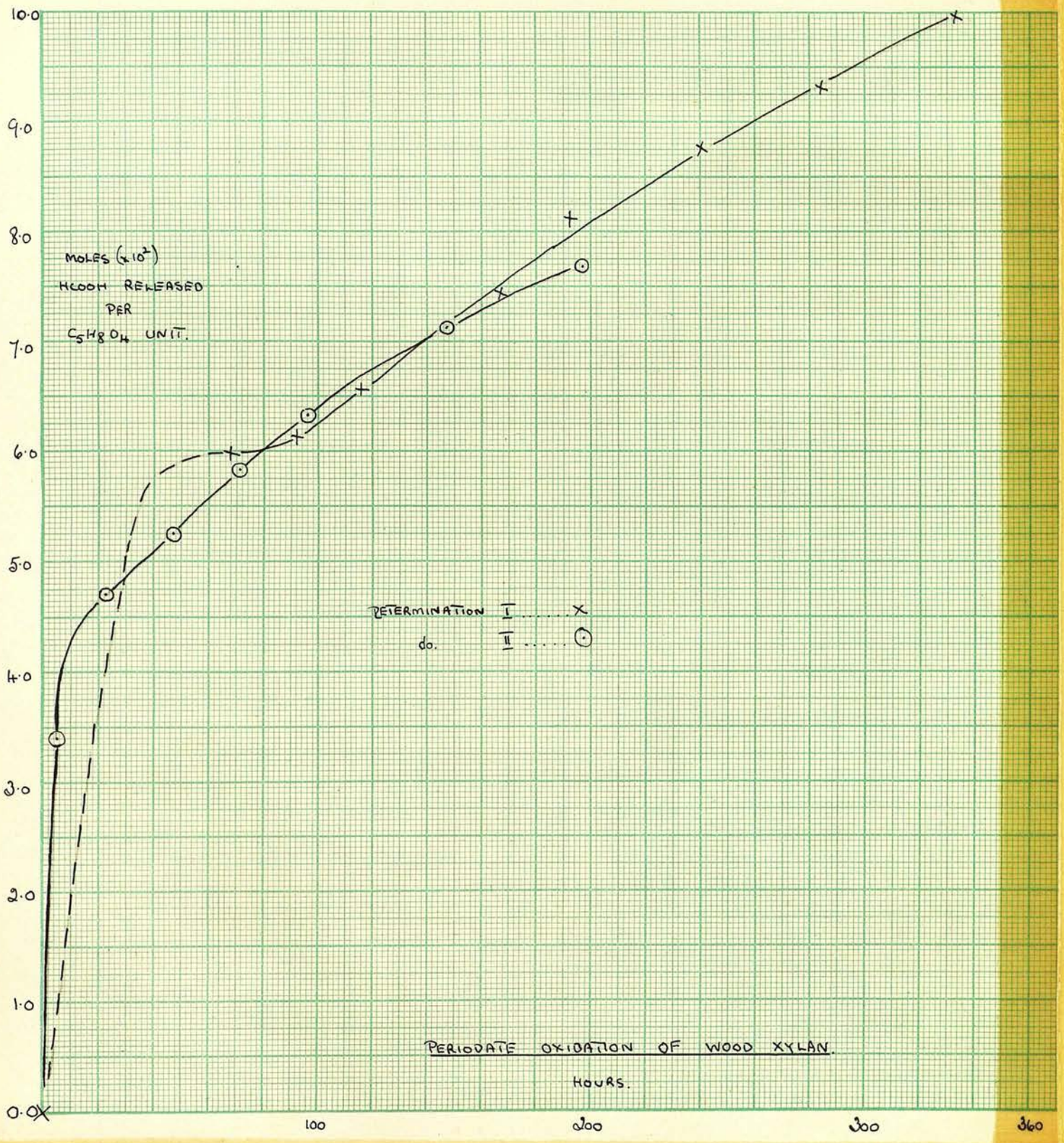
A number of small bottles containing hemicellulose A were set up as for the periodate uptake of straw xylan (p.53), (168).

Table 5.

Time (hrs.	49	68	92	116	164	260
Mols. periodate consumed per $C_5H_8O_4$ unit.	0.92	0.98	0.99	1.04	1.02	1.11

2. Formic acid release (11).

The method outlined on p.52 was used. Two determinations were carried out to check on a possible stationary value at 6×10^{-2} moles, (see graph). Results are tabulated overleaf.



PERIODATE OXIDATION OF WOOD XYLAN.

Table 6.

Time (hrs.)	68	92	116	167	192	240	284	333
10^2 x Moles formic acid/ $C_5H_8O_4$ unit.	5.98	6.12	6.56	7.42	8.12	8.74	9.31	9.94

Table 7.

Time (hrs.)	5	23	47	71	96	147	197
10^2 x Moles formic acid/ $C_5H_8O_4$ unit.	3.40	4.70	5.25	5.82	6.32	7.12	7.68

Hemicellulose A Acetate (173).

Hemicellulose A (2 g.) was dispersed in formamide (60 ml.) at 60° for 45 minutes, and the mixture was shaken at room temperature for 16 hours. Dry pyridine (56 ml.) was then added at 40° and acetic anhydride (22 ml.) was added slowly at 45° with stirring, during a period of 30 minutes. The solution was then shaken in the dark at room temperature for a further 16 hours, after which time it had set to a dark green gel.

To effect solution of the gel, benzene (200 ml.) was added with vigorous shaking at 40°. Partial solution only was accomplished. The clear solution was decanted and poured into ice water (3 l.) with vigorous stirring. The benzene gave a two phase liquid mixture, with the precipitated acetate remaining largely as slime in the benzene layer. To the remaining gel was added a little m-cresol, which caused complete solution. This solution was successfully precipitated in the ice water (3 l.) as a brown, flocculent precipitate

the aqueous mixture after precipitation being pH 7. The precipitate was continually washed with water at the centrifuge until traces of pyridine had been removed. On addition of ethanol the precipitate gelatinised. Ethanol was therefore removed, the precipitate suspended in cold water with vigorous stirring and freeze dried to a pale brown powder. A second acetylation was performed as above on the product. Formamide was not used.

Acetyl content (see p.91), 39.8%.

Calc. for xylan diacetate 39.8%.

Methylation.

Hemicellulose A (20 g.) was methylated (x 7) under nitrogen in the manner described for straw xylan (p.58). The partly methylated xylan was removed by filtration, after neutralisation of the mixture with sulphuric acid (2N), and the solid was washed free from sodium sulphate, dissolved in acetone (300 ml.) and methylated a further 5 times.

The final product was found to be slightly soluble in hot water giving an opalescent solution on cooling. It was therefore decided to remove remaining traces of sulphate by extraction of the xylan with chloroform. On addition of chloroform, however, the solid formed a gel. The swollen solid was therefore dialysed against tap water in a cellophane bag until free from sulphate. Solid was then separated from aqueous solution at the centrifuge and solvent was removed from both at 30°/15 mm. Solids obtained were combined.

The material was partially soluble in methyl iodide to give a white milky suspension on heating. Addition of small quantities of acetone, methanol or chloroform did not markedly increase the solubility.

After boiling under reflux with methyl iodide (200 ml.) for 3 hours, solvent was removed at 30°/15 mm., and the solid was suspended in acetone (500 ml.) and given a further 5 methylations with dimethyl sulphate (180 ml.) and sodium hydroxide as before. When reclaiming the methylated product dialysis was found to be unnecessary, since the product now appeared to be insoluble in water. Sulphate was therefore removed by washing the solid with water.

The dry product was suspended in methyl iodide (175 ml.), methanol (1 ml.) and chloroform (2 ml.) and boiled under reflux (1 hour). Silver oxide (100 g.) was then added at intervals over 48 hours, the mixture being kept at the boiling point. After this time, the suspension cleared to a solution. The methylated material was regained by extraction of the silver residues in the normal way. Yield, 16.6 g.

The methylated material was now completely soluble in methyl iodide. Two further Purdie methylations were given using methyl iodide (200 ml.) and silver oxide (100 g.). Yield, 14.65 g. Methoxyl, 38.2%

Fractionation.

The solvents used were the same as for straw xylan (p.59). In each case, except No.1, the chloroform was added first in sufficient quantity to effect complete

solution. The requisite amount of light petroleum was then added, and the mixture heated under reflux for 30 minutes. Each solvent mixture was given two such treatments, (300 ml.).

Table 8.

Fraction No.	Chloroform: light petroleum.	Weight.	Yield.
1.	0:100	0.20 g.	1.36%
2.	10:90	0.40 g.	2.74%
3.	20:80	0.25 g.	1.50%
4.	25:75	1.10 g.	7.50%
5.	30:70	12.70 g.	86.75%

Solvent was removed at 30°/15 mm. Fraction 5 yielded a syrup which was dissolved in a little chloroform and precipitated in light petroleum (2 l.). On separating the solid at the centrifuge, a white powder was obtained. This had OMe, 38.6%; $[\alpha]_{\text{D}}^{19}$ - 75° (c, 0.234 in chloroform) and $[\alpha]_{\text{D}}^{18}$ - 113° (c, 1.25 in m-cresol).

Hydrolysis of Methylated Xylan, and Separation of Fragments. By cellulose column.

Fraction 5 (5 g.) was boiled under reflux with methanolic hydrogen chloride (500 ml.; 1%). After 3 hours boiling some solid remained. The concentration of acid was therefore increased to 1½% (530 ml.). $[\alpha]_{\text{D}}^{20}$ 71 (9 hrs.), 74 (10 hrs.), 74 (12 hrs.). The acid was then neutralised with silver carbonate, the mixture filtered, and the solid was washed with methanol and with water. The filtrate and washings were reduced to a mobile syrup at 18 mm., which was rehydrolysed with

hydrochloric acid (200 ml.;1N) at 95° to constant rotation. $[\alpha]_D^{20}$ 37° (1 hr.) ⟶ 41° (12 hrs.). The acid was neutralised with silver carbonate, the mixture filtered, and silver ions were removed with hydrogen sulphide. After concentration at 40°/18 mm., the syrup obtained was redissolved in a little water (10 ml.), and filtered. Water was removed as before, yielding a clear yellow syrup (4.541 g.).

From the yield obtained, it was decided that some sugar might have been lost in the silver residues. These were therefore thoroughly extracted as follows:-

(a) The silver residue from the methanolysis neutralisation was shaken with several batches of cold hydrochloric acid (0.1N), filtered, and allowed to stand overnight.

(b) The silver residue from neutralisation of the aqueous hydrolysate was shaken thoroughly with distilled water, and filtered. The extracts were combined, neutralised, and silver ions were removed as before. By this means a further 0.190 g. of syrup was recovered. Total yield, 4.751 g.

Separation of methylated sugars.

The syrup (3.920 g.) was dissolved in a little of the solvent used (see below) and applied to the top of a cellulose column (90 x 4 cms.). The eluate was collected and purified as described previously. The eluting solvent used was purified light petroleum (b.p. 100-120°) - purified n-butanol, 65:35, saturated with water. It was found that, due to the high concentration of uronic acid present, a complete

separation was not achieved, uronic acid accompanying all but the first two fractions. A water wash was therefore applied to remove the last fractions for later re-separation. Recovery was 91%.

Table 9.

Fraction.	Sugar.	Weight.	Eluant at column head.
1A.	2:3-dimethyl methyl β D xyloside.	0.022 g.	Butanol:light pet. 65/35 sat. H_2O.
2A.	2:3:4-trimethyl xylose.	0.046 g.	do.
3A.	Uronic acid.	0.056 g.	do.
4A.	2:3-dimethyl xylose, Uronic acid.	0.121 g.	Water.
5A.	2:3-dimethyl xylose, Monomethyl xylose, Uronic acid.	2.988 g.	do.
6A.	Uronic acid.	0.321 g.	do.

Fraction 4A.

It was attempted to separate this fraction into its components. The fraction was dissolved in a little water (10-15 ml.), and solid barium carbonate (1 g.) was added. After standing for 24 hours at room temperature, the mixture was exhaustively extracted with chloroform in a liquid extractor (24 hrs.). The aqueous extract was filtered free from barium carbonate, and the filtrate was treated with Amberlite resin IR 100(H) and evaporated to dryness at 40°/15 mm.

The brown glassy solid obtained (63 mg.) was shown to be uronic acid free from the dimethyl sugar when examined paper chromatographically. The chloroform

extract was evaporated at 15 mm. to yield a syrup (35 mg.) which was largely dimethyl xylose with a trace of uronic acid. The syrup was added to fraction 5A (below).

Fraction 5A. (3.023 g.)

The syrup was dissolved in a little n-butanol: ethanol:water:ammonia (40:10:50:1) solvent and applied to a cellulose column (60 x 4 cms.), using this solvent as eluant. The collected fractions were treated as before.

A complete separation was again not achieved due to the occurrence of a sharp drop in temperature during running. This caused the solvent in the column to separate into two phases, i.e. to become supersaturated with respect to water. Recovery was 91%.

Table 10.

Fraction.	Sugar.	Weight.	Eluant.
1B.	2:3-dimethyl xylose.	2.108 g.	n-butanol:ethanol: water:ammonia.
2B	2:3-dimethyl xylose, Monomethyl xylose.	0.390 g.	do.
3B	Uronic acid.	0.242 g.	Water.

After the mono and dimethyl xylose had all been eluted a water wash was applied to remove the uronic acid from the column. This was purified, as were the other fractions, after which the syrup was dissolved in a little methanol (1 ml.), filtered, and evaporated at 40°/15 mm. to a semi-solid syrup. The syrup was extremely soluble in methanol and in water.

Fraction 2B. (0.390 g.)

The syrup was separated on a cellulose column

(50 x 3 cms.), fractions being collected and purified as before. The eluant used was purified n-butanol: light petroleum (b.p. 100-120°), 30:70, saturated with water. Recovery was 93-4%.

Table 11.

Fraction.	Sugar.	Weight.
1C.	2:3-dimethyl xylose.	0.112 g.
2C.	Monomethyl xylose.	0.252 g.

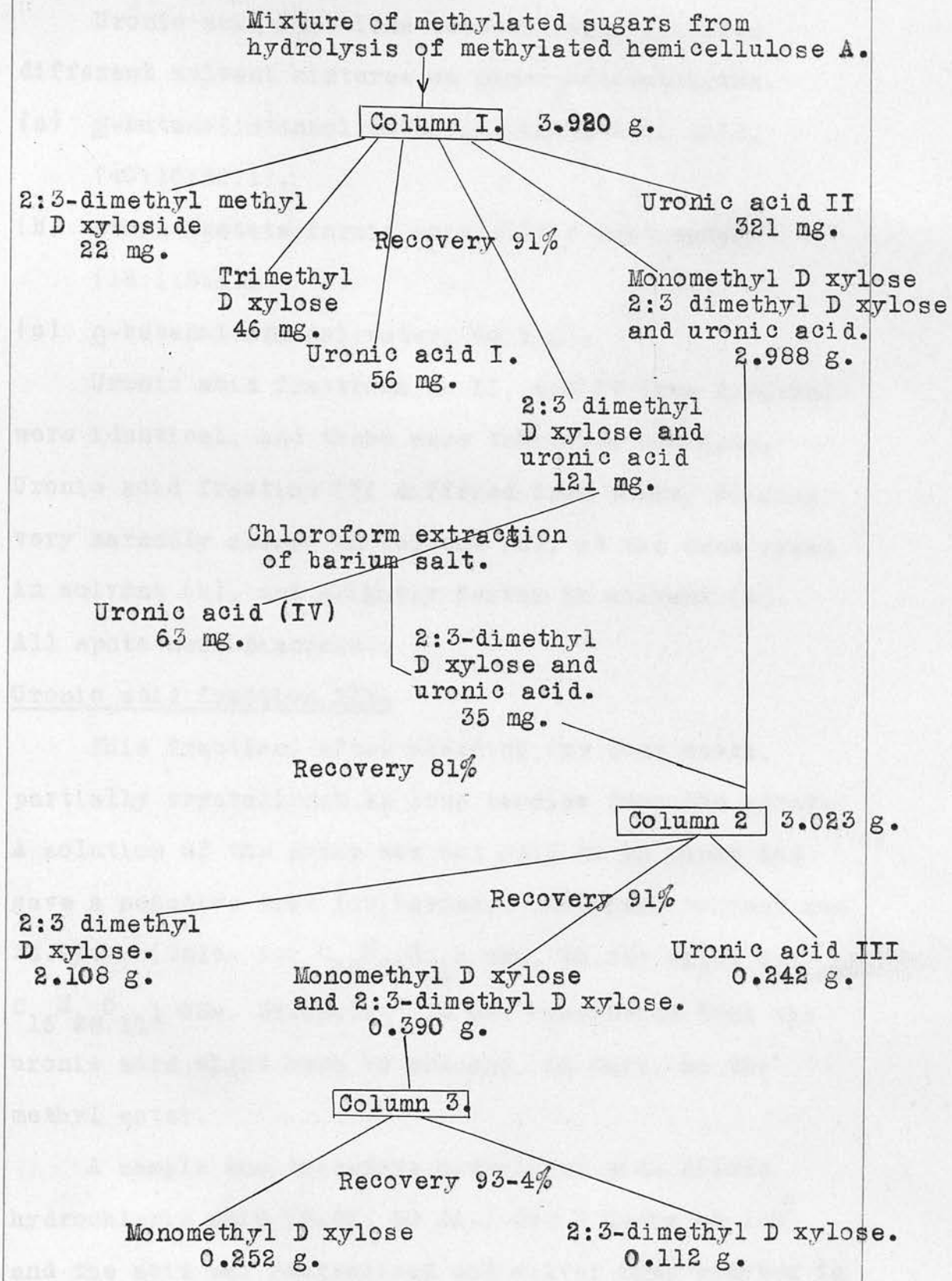
Samples of the same sugar from various stages of the above separation were combined.

Separation Summary.

Table 12.

Sugar.	Pure material obtained.	In hydrolysate. Calc.wt.before sepn.	Mol.%.
2:3:4-trimethyl D xylose.	46.0 mg.	50.9 mg.	1.34%
2:3-dimethyl D xylose.	2.240 g.	2.7501 g.	78.16%
Monomethyl D xylose.	252 mg.	327 mg.	10.08%
Tetramethyl aldobiuronic acid.	682 mg.	792 mg.	10.49%

Separation Summary.



Uronic Acid Fractions.

Uronic acid fractions were compared in three different solvent mixtures on paper chromatograms.

(a) n-butanol:ethanol:water:glacial acetic acid, (40:10:50:1).

(b) ethyl acetate:formic acid:acetic acid:water, (18:1:3:4).

(c) n-butanol:ethanol:water, (4:1:5).

Uronic acid fractions I, II, and IV (see diagram) were identical, and these were therefore combined. Uronic acid fraction III differed from these, running very markedly slower in solvent (a), at the same speed in solvent (b), and slightly faster in solvent (c). All spots were discrete.

Uronic acid fraction III.

This fraction, after standing for some weeks, partially crystallised in long needles from the syrup. A solution of the syrup was not acid to pH paper and gave a negative test for barium. Methoxyl content was 34.9%. (Calc. for $C_{15}H_{26}O_{11}$; OMe, 32.5%; calc. for $C_{16}H_{28}O_{11}$; OMe, 39.2%.). It was considered that the uronic acid might here be present, in part, as the methyl ester.

A sample was therefore hydrolysed with dilute hydrochloric acid (0.5N; 50 ml.) for 3 hours at 100°, and the acid was neutralised and silver ions removed in the usual way. The product, compared paper chromatographically with the other uronic acid fractions was shown to be identical.

Demethylation. (171).

Samples (10 mg.) of both uronic acid fractions (I + IV + II) and III were demethylated with hydrobromic acid as described previously. The neutral products were examined paper chromatographically in n-butanol:ethanol:water (4:1:5) and n-butanol:benzene:pyridine:water (5:1:3:3) solvents against the relevant standards. Xylose, present in both samples, was the only simple sugar detected. A uronic acid, having in the former solvent an R_G of 0.03, was also detected in both samples.

The remaining quantity of uronic acid fraction III was therefore hydrolysed as for the sample above, and the sugar was regenerated to give a fraction identical with the other uronic acid fractions (I + IV + II).

EXAMINATION OF COMBINED URONIC ACID FRACTIONS.

The combined fractions had equivalent, 399; OMe, 32.3%. (Calc. for $C_{15}H_{26}O_{11}$, equivalent, 382; OMe 32.5%; calc. for $C_{22}H_{37}O_{15}$, equivalent 541; OMe, 34.2%), and $[\alpha]_D^{19}$ + 51.5° (c, 0.78 in water).

(a) Reduction (182).

A sample (210 mg.) of uronic acid was boiled under reflux with methanolic hydrogen chloride (50 ml.;1%) for 6 hours, neutralised with silver carbonate and filtered after standing overnight. The neutral solution was evaporated to a dry syrup at 15 mm., the syrup being then dissolved in dry ether (75 ml.) and lithium aluminium hydride (200 mg.) was added over 3 hours. The mixture was, for this time, heated under reflux. Excess

of lithium aluminium hydride was then destroyed carefully with water, and the solution was just acidified to methyl orange with sulphuric acid (2N). The mixture was reduced to half volume at 40°/15 mm., and extracted with chloroform (50 ml. x 3). The chloroform extract was evaporated at 15 mm., and the resulting syrup was hydrolysed with hydrochloric acid (50 ml.;0.5N) for 7 hours at 100°. After neutralisation with silver carbonate, soluble silver was removed with hydrogen sulphide. The product was examined on a paper chromatogram in n-butanol:benzene:pyridine:water (5:1:3:3) solvent against standard 2-methyl xylose, 3-methyl xylose and 2:3:4-trimethyl glucose. Sugars corresponding to 3-methyl xylose and 2:3:4-trimethyl glucose were observed. A trace of sugar of low R_G (0.10) was also seen on very heavy spotting. The product, which was acid even after aeration, was neutralised with barium carbonate, filtered, and examined in n-butanol:ethanol:water (4:1:5) solvent. 3-methyl xylose and 2:3:4-trimethyl glucose were found to be present in approximately equal quantities, together with traces of the sugar of low R_G (0.10). This, from colour and position, was assumed to be a disaccharide. A trace of 2:3-dimethyl xylose which was thought to be present, could not be decisively confirmed on heavy spotting.

The aqueous layer from the chloroform extract was filtered, neutralised with silver carbonate, reduced to small volume (2-3 ml.), filtered, and hydrolysed with

sulphuric acid (10 ml.; 1N) for five hours at 100°. The mixture was cooled, neutralised with barium carbonate, filtered, and evaporated at 15 mm. to a syrup. The dry syrup was dissolved in dry methanol (1 ml.) and crystals (white, lithium sulphate ?) were filtered off. The filtrate, with methanol removed, was dissolved in a little water and treated, separately, with Amberlite resins IR 120(H) and IR 4B(OH). Amberlite IR 100(H) was found to be unsuitable for use here, the mixture turning alkaline (pH 9) instead of acid when this resin was used. On examination of the solution on a paper chromatogram, there was detected, in small amount, the same sugars as were present in the syrup from the chloroform extract, in approximately the same proportions. Both products were, therefore, combined, reduced at 15 mm. to small volume, filtered, and finally reduced to a syrup (163 mg.).

Separation and Identification of component sugars.

The syrup (163 mg.) was separated into its components by chromatography on a long, thick, Whatman No.1 filter sheet using n-butanol:ethanol:water (4:1:5) solvent.

The paper was irrigated before use with hot water, and dried. Side reference strips were spotted with standard sugars to increase the recovery. Monomethyl xylose was extracted from the paper in a Soxhlet extractor with water (2 hrs.), and the trimethyl glucose was extracted with chloroform. Development of the dried strips with aniline oxalate solution after extraction showed that all sugar had been extracted.

The extracts were reduced to syrups at 40°/15 mm., dissolved in the minimum amount of cold water, filtered through a washed bed of "Filter-cel", evaporated to small volume at 15 mm., refiltered, and finally evaporated to syrups at 40°/15 mm.

Yields obtained were (a) monomethyl xylose, 67 mg., (b) trimethyl glucose, 79 mg., giving a molar ratio for these sugars of 1.15;1.0.

Fraction (a).

Fraction (a) had OMe, 18.5%. (Calc. for $C_6H_{12}O_5$, OMe, 18.9%).

This fraction gave a discrete spot on chromatographic examination. A long paper chromatogram (66 hours; 20°) in n-butanol:ethanol:water (4:1:5) solvent, developed with aniline oxalate solution (saturated) gave, in comparison with standard 2-methyl and 3-methyl xylose, indecisive results since these sugars have closely similar R_G values. The colour of fraction (a) on development with aniline oxalate solution appeared similar to that of standard 3-methyl xylose, being brown-pink whilst standard 2-methyl xylose appeared purple-pink.

On development of such a chromatogram with a spray of p. anisidine hydrochloride (2%) in n-butanol, fraction (a) was clearly seen to be 3-methyl xylose. With the p. anisidine reagent this sugar gives a yellow-brown whilst 2-methyl xylose gives a purple colour. The colour differences are heightened under ultra violet light.

An ionophoretogram paper was prepared using borate

buffer, pH 9.97 (218), and a potential difference of 400 volts for 4 hours at room temperature. Chlorobenzene was employed as the inert solvent (217). On developing the paper with a spray of aniline oxalate (saturated) and glacial acetic acid (5/1, v/v) in the air oven at 100°, fraction (a) and standard 3-methyl xylose gave a single, discrete purple spot, distant 10.1 cms. from the starting line. Standard 2-methyl xylose gave a discrete brown spot 5.7 cms. from the starting line with, in addition, a trace of the purple spot at 10.1 cms. indicating the presence of a small quantity of 3-methyl xylose impurity in the 2-methyl xylose standard.

The syrupy fraction (a) was seeded with a crystal of 3-methyl xylose (200) and, after standing at 0°, partially crystallised in colourless crystals.

The crystals had $[\alpha]_D^{20}$ + 19.5° (c, 0.514 in water), and m.p. 86-8° after drying from adhering syrup on a porous tile. The m.p. was undepressed by admixture with authentic crystalline 3-methyl D xylose. Recrystallisation of the sample was attempted from a number of solvents but crystals could not be induced to re-form from the syrup.

Preparation of 3-methyl D xylosazone from Fraction (a).

A sample (5.1 mg.) of fraction (a) was taken with which to form the osazone. The sugar was heated in a small tube with phenyl hydrazine hydrochloride (25 mg.), hydrated sodium acetate (70 mg.), water (1 ml.), and a trace of sodium bisulphite at 95° on a water bath for 35 minutes. After this time, the clear solution was

cooled and a yellow mass of crystals was immediately deposited. The mass was filtered, washed thoroughly with cold water and dried on the water pump. The filtrate was re-heated for 10 minutes on the water bath at 95°, and a second crop of identical crystals was obtained in good yield, on cooling the mixture. These were treated in the same manner as above.

The dry yellow needles had m.p. 172°, (dropping to 169-70° after storage for 4 days). The crystals did not, on admixture with authentic 3-methyl D xylosazone crystals, cause depression in m.p. of the latter. However, a mixture of authentic D xylosazone with the crystals obtained above gave a markedly low m.p. (130-151°).

Fraction (a) was thus pure 3-methyl D xylose.

Fraction (b).

Fraction (b) had OMe, 40.6%. (Calc. for $C_9H_{18}O_6$, OMe, 41.8%).

This fraction was boiled under reflux with methanolic hydrogen chloride (10 ml.; 1%) until the mixture was no longer reducing to Fehling's solution. Acid was then neutralised with silver carbonate, and silver ions were removed by filtration of the liquid after standing (12 hours). Methanol was removed at 15 mm., and the resulting syrup, seeded with authentic 2:3:4-trimethyl methyl β D glucoside, was allowed to crystallise at 0°. A sample of the white needles, with adhering syrup, was placed on a clean, dry tile, and after one week the crystals were removed. They had m.p. 88-92°, raised to 92-3° on recrystallisation from dry ether, undepressed

by admixture with authentic 2:3:4-trimethyl methyl β D glucoside.

Fraction (b) was thus 2:3:4-trimethyl D glucose.

(b) Methylation, and reduction of the Combined Uronic Acid Fraction, (182).

A sample (111 mg.) of the combined uronic acid fraction was boiled under reflux with methanolic hydrogen chloride (22 ml.;1.8%) for 5 hours, neutralised with silver carbonate,and filtered, after standing.

The filtrate was evaporated to a syrup at 15 mm., dissolved in neutral methyl iodide (15 ml.), and heated at 60° under reflux with continual addition of quantities of silver oxide to a total of 6-7 g., (8 hrs.). The mixture was filtered and the residue thoroughly extracted with hot chloroform. The combined filtrate was evaporated to a syrup at 15 mm., redissolved in methyl iodide (15 ml.), and a second Purdie methylation was given as before.

After extraction and filtration, the solvent was removed at 15 mm., yielding a clear, yellow syrup which was dissolved in dry ether (75 ml.), and treated with lithium aluminium hydride (200 mg.) with heating under reflux at 30° (5 hrs.). Excess of lithium aluminium hydride was destroyed with water. The mixture was then acidified with sulphuric acid (2N) to methyl red-methylene blue (183), and ether was removed at 15 mm. The reduced product was then extracted exhaustively with hot chloroform, and the extract was reduced to a syrup at 35°/15 mm.

The syrup was hydrolysed with hydrochloric acid

(25 ml.;0.5N) for 6-7 hours at 100°, neutralised with silver carbonate, filtered, and soluble silver was precipitated with hydrogen sulphide. After further filtration, the mixture was evaporated at 40°/15 mm. to a syrup (56 mg.).

A chromatogram in n-butanol:ethanol:water (4:1:5) showed 2:3:4-trimethyl glucose and 3:4-dimethyl xylose to be present in approximately equal quantities, with a little 3-methyl xylose, and a trace of 2:3:4-trimethyl xylose. Standard mono and dimethyl xyloses were run. A long chromatogram in the same solvent, developed with a spray of p. anisidine hydrochloride (2%) in n-butanol showed the dimethyl xylose to be the 3:4- isomer. No 2:3-dimethyl xylose was observed. Although the R_G values of these sugars are closely similar, distinctly different colours are obtained with the above spray reagent; yellow-brown and red-purple, respectively. An orange colour is given with 3:5-dimethyl xylose. The 2:3:4-trimethyl glucose appeared as a yellow-green spot, darkening on standing. Using the above spray reagent, a small quantity of 2:3-dimethyl xylose impurity in the standard 3:4-dimethyl xylose was easily discernible.

An attempt was made to detect formaldehyde using periodate, glycol, and Schiff's reagent as sprays on paper chromatograms. Unambiguous results could not, however, be obtained.

Separation of the methylated mixture.

The remaining syrup (circ. 45 mg.), was separated

on a long, thick (3MM) Whatman No.1 filter sheet in n-butanol:ethanol:water (4:1:5).

Sugars from the strips were eluted with boiling water in the normal manner, and were estimated with alkaline hypoiodite. To the sugar solutions in stoppered boiling tubes was added phosphate buffer, pH 11.4 (2 ml.), and iodine (1 ml.;0.1N). After 4-5 hours in the dark, iodine consumption was estimated against 0.02306N sodium thiosulphate, samples being just acidified with sulphuric acid (2N) before titration. A blank was run and, in addition, a control sample of 2:3-dimethyl xylose (circ. 20 mg.). The latter was for use in the formaldehyde experiment below. No starch solution was used at the end point.

Results gave titre figures for 2:3:4-trimethyl glucose, 3:4-dimethyl xylose, and 3-methyl xylose as 1.55; 1.48; 0.74 ml. respectively, giving a molar ratio of 1.0: 0.95: 0.48. The trace of 2:3:4-trimethyl xylose present was too small for estimation.

To the colourless solutions, including the blank, and the oxidised 2:3-dimethyl xylose control which had been just neutralised with sodium thiosulphate, was added sodium metaperiodate solution (5 ml.;0.25M). This caused an immediate heavy precipitation of iodine. After 45 hours in the dark, stoppered, at room temperature, samples of each were removed, excess periodate was destroyed with sodium arsenite (0.1N) and a colorimetric test for formaldehyde was performed using potassium ferricyanide and phenyl hydrazine hydrochloride (176).

A large excess of arsenite was avoided.

Results were positive for 2:3:4-trimethyl glucose and the 2:3-dimethyl xylose control; negative for the 3:4-dimethyl xylose and the blank. A clear red or red-brown colour was taken as positive, a clear yellow or yellow-green as negative. The experiment was repeated several times with successive samples. An inconclusive chocolate coloured precipitate may be obtained if interfering ions are present in excess.

EXAMINATION OF OTHER FRACTIONS.

Fraction 1A.

This crystalline substance (22 mg.), examined on a paper chromatogram in n-butanol:ethanol:water (4:1:5) solvent had an R_G value slightly faster than 2:3:4-trimethyl xylose. On heavy spotting, and development with saturated aniline oxalate solution, it gave an ill defined brown spot.

The colourless needle crystals had m.p. 63°. $[\alpha]_D^{16}$ -47.3° (c, 0.148 in water).

A sample was hydrolysed with hydrochloric acid (5 ml.;0.5N) for 3 hours at 100°, and the sugar was recovered in the usual way. On examination of the resulting syrup on a paper chromatogram, a spot identical with that given by standard 2:3-dimethyl xylose, R_G 0.74, was obtained. No other sugar was detected.

A sample of standard 2:3-dimethyl xylose (circ. 50 mg.) was boiled under reflux with methanolic hydrogen chloride (10 ml.;1%) until the mixture was no longer reducing to Fehling's solution. Acid was neutralised with

silver carbonate, and the filtrate was evaporated to a syrup at 35°/15 mm.

The syrup was seeded with a crystal of fraction 1A and, on storing overnight at 0°, identical crystals separated. On attempting to tile a sample of these crystals with a view to obtaining a mixed melting point with fraction 1A, it was found that the melting point of the crystals in the $\alpha\beta$ mixture was below room temperature. Repeated attempts to separate crystals from syrup using chilled solvents were unsuccessful.

Fraction 2A.(46 mg.)

This fraction crystallised immediately on seeding with authentic crystalline 2:3:4-trimethyl D xylose. The brown, crystalline mass obtained was washed quickly with a little purified light petroleum (b.p. 60-80°) and the liquid was removed by suction. Recrystallisation from ether gave large, colourless crystals m.p. 89°, undepressed on admixture with authentic crystalline 2:3:4-trimethyl D xylose.

The crystals had $[\alpha]_D^{20}$ + 22° (c, 0.9 in water); OMe, 48.2%. (Calc. for $C_8H_{16}O_5$, OMe, 48.4%). Hypoiodite oxidation buffered at pH 11.4 indicated 99% pure aldopentose, and chromatographic examination showed a discrete, pink spot having R_G 0.94.

The derived anilide (see p.71) had m.p. 98-9° alone or mixed with an authentic specimen of 2:3:4-trimethyl D xylose anilide.

Fractions 1B and 1C.

The clear, yellow syrup crystallised on seeding

with authentic crystalline 2:3-dimethyl D xylose and storing in a refrigerator, the crystallisation proceeding extremely slowly.

The fraction gave a discrete spot, R_G 0.74, on chromatographic examination and had $[\alpha]_D^{15}$ + 22.7° (c, 1.1 in water); OMe, 34.5%. (Calc. for $C_7H_{14}O_5$, OMe, 34.8%). Purity by buffered alkaline hypoiodite was 97-8%.

The crystals had m.p. 78° after washing, on a porous tile with purified petroleum ether (b.p. 100-120°), free from syrup. The m.p. was undepressed by admixture with authentic 2:3-dimethyl D xylose.

The derived anilide (see p.74) had m.p. 123° alone or mixed with authentic crystalline 2:3-dimethyl D xylose anilide recrystallised from ethyl acetate-light petroleum.

Fraction 2C. (252 mg.)

The yellow, viscous syrup had OMe, 18.5%. (Calc. for $C_6H_{12}O_5$, OMe, 18.9%); $[\alpha]_D^{20}$ + 17° (c, 0.8 in water), and a purity by buffered alkaline hypoiodite of 97%.

On paper chromatographic examination in various solvents a single discrete spot was given. A long (66 hours) paper in n-butanol:ethanol:water:ammonia (40:10:50:1) solvent, developed with saturated aniline oxalate solution, gave a single spot of R_G 0.38, of colour intermediate between that of standard 2-methyl and 3-methyl xylose - these being purple-pink and brown-pink, respectively.

On development of such a chromatogram with a spray of p. anisidine in n-butanol, fraction 2C appeared identical in colour with standard 3-methyl xylose (yellow-brown).

Standard 2-methyl xylose gave a purple spot on development with this reagent.

An ionophoretogram paper was prepared using Whatman No.1 filter sheet impregnated with borate buffer, pH 9.97, and a potential difference of 400 volts for 4 hours at room temperature. Chlorobenzene was employed as the inert organic liquid (217). The ionophoretogram paper was dried in air and developed with a spray of saturated aniline oxalate solution and glacial acetic acid (5/1, v/v) in an air oven at 100°. This showed fraction 2C to be a mixture of 3-methyl and 2-methyl xylose estimated visually, against standards, at 20-25:1. There is a wide difference between the positions and colours of the spots given by standard 2-methyl and standard 3-methyl xylose under these conditions (see p.114).

The syrupy fraction 2C was set aside but did not crystallise, even on seeding with crystalline 3-methyl xylose and after long storage.

The anilide of fraction 2C was prepared as has been described for previous fractions. In the absence of an authentic, crystalline specimen of 3-methyl D xylose anilide, the syrup obtained was seeded with a minute quantity of crystalline 2-methyl D xylose anilide, and long crystals of 3-methyl D xylose anilide were obtained.

After washing with purified light petroleum (b.p. 100-120°) to remove aniline and drying, these crystals had m.p. 136°.

HYDROLYSIS OF UNMETHYLATED HEMICELLULOSE A, AND EXAMINATION OF THE ACIDIC FRACTION.

Hemicellulose A (10 g.) was heated on the boiling water bath with sulphuric acid (100 ml.;1N) for 5-6 hrs. After cooling, the clear supernatant liquid was decanted from a black residue, neutralised with excess of barium carbonate, filtered, and set aside (I).

The residue was again treated with sulphuric acid (100 ml.;1N) for 4 hrs. under reflux, and filtered. The remaining solid was washed, filtrate and washings being neutralised with barium carbonate, and the neutral filtrate free from barium carbonate was added to I. This liquid was concentrated to one quarter volume, refiltered and finally concentrated to a syrup at 40°/15 mm.

The syrup was poured dropwise into dry methanol (150 ml.) with vigorous mechanical stirring. After a short time (20 mins.), a small precipitate (circ. 100 mg.) flocculated and settled. The supernatant methanol (II) was removed, and the precipitate was washed and dried to a brown, curdy solid (III).

The solid (III) was dissolved in the minimum quantity of water, filtered, and reprecipitated by the addition, to it, of dry methanol in excess. The precipitate (IV) was removed from methanol as before, washed with methanol several times, and dried. The methanol and washings were added to (II).

Chromatographic examination of the solid (IV) in n-butanol:ethanol:water:ammonia (40:10:50:1) showed some uronic acid, and some free xylose, to be present. The dark brown colour of the solid indicated a high ash content.

To the liquid (II) was added the same volume of dry ethanol. An immediate precipitate of a cream coloured solid which was obtained was washed and dried. The supernatant liquid was evaporated at 15 mm. to a syrup and added dropwise, with stirring, to ethanol. A further precipitate of a cream coloured solid was washed, separated and dried. Chromatographic examination of both solids obtained showed uronic acid and xylose in approximately equal proportions.

The cream coloured solids were mixed, dissolved in the minimum of cold water, and reprecipitated by the addition of excess of methanol, washed, and dried. This procedure was repeated several times. The approximate proportion of free xylose to uronic acid, as judged by qualitative paper chromatograms, remained the same throughout.

Yield, 1.114 g. OMe, 4.95% (Calc. for barium monomethyl aldobiuronate, OMe, 7.6%). Equiv., 384. (Calc. for $C_{12}H_{20}O_{11}$, equiv., 340).

The final ethanol solution, evaporated to a syrup at 15 mm. showed xylose predominantly on a paper chromatogram, with only a trace of uronic acid.

Note: The sample of uronic acid on which the methoxyl content was determined was dissolved in water, and evaporated to dryness at 40°/15 mm., before weighing.

Attempted preparation of the Amide of 4-methyl methyl D glucuronoside.

The crude barium uronate (50 mg.) obtained above was subjected to vigorous methanolysis with methanolic

hydrogen chloride (5 ml.;11%) for 20 hours in a sealed tube at 95°. The solution was neutralised with silver carbonate, filtered, and reduced at 15 mm. to a syrup.

The syrup was treated with saturated methanolic ammonia (3 ml.) at 0° for 24 hours, after which the solvent was removed at 15 mm., and the resulting syrup was stored in a refrigerator. No crystals separated.

Attempted preparation of Aldobiuronamide methyl glycoside.

Barium salt (50 mg.) was heated under reflux with methanolic hydrogen chloride (10 ml.;1%) for 6 hours at 60°, neutralised with silver carbonate, and filtered. Methanol was removed at 15 mm., and the syrup was treated with saturated methanolic ammonia (2 ml.) for 24 hours at 0°. Solvent was then removed at 15 mm., and the syrup was stored at 0°. No crystals separated.

Hydrolysis of the Crude Barium Uronate, (see also p. 127).

A sample (70 mg.) of crude barium uronate was subjected to vigorous methanolysis in a sealed tube with methanolic hydrogen chloride (5 ml.;16%) for 20 hours at 70-80°. The solution was neutralised with silver carbonate, filtered and reduced at 15 mm. to a syrup (V).

A sample of the syrup (V) was hydrolysed with hydrochloric acid (0.5N; 2 ml.) at 100° for 4 hours, neutralised and reduced to a syrup in the normal manner. This was then examined paper chromatographically:

(a) using n-butanol:ethanol:water:acetic acid (40:10:50:1).

Xylose, and a little 4-methyl glucuronic acid together with some glucuronic acid was observed. The

presence of glucurone could not be confirmed. In addition, a faint yellow spot was detected travelling 26.2 cms. when xylose had moved 12.5 cms. The relevant standards were run, and the developer used was aniline oxalate solution (saturated).

(b) using ethyl acetate:acetic acid:formic acid:water (18:3:1:4).

Xylose was observed, and also an unidentified sugar travelling 40.2 cms. when standard glucurone had moved 32.0 cms. Glucuronic acid (29.3 cms.) was observed, together with the orange colour associated with 4-methyl glucuronic acid travelling slightly ahead of xylose. Relevant standards were again run, and the developer used was aniline oxalate solution.

Reduction of Crude Barium Uronate.

The syrup (V) was dissolved as far as possible in dry ether (10 ml.) and treated, under reflux, with lithium aluminium hydride (100 mg.;3 hrs.). After destruction of the excess of hydride with water, the solution was acidified with sulphuric acid (2N), filtered, and reduced at 15 mm. to small volume (5 ml.). The solution was treated repeatedly with alternate batch quantities of Amberlite resins IR 120(H) and IR 4B(OH) to remove inorganic ions, finally being concentrated to a syrup.

Paper chromatographic examination of the syrup in n-butanol:ethanol:water (4:1:5) solvent showed xylose to be present, with a definite trace of glucose, and possibly a trace of glucurone. A faster spot, R_G 0.57 (yellow), and a spot, R_G 0.47 (faint brown) were observed,

both of which travelled at approximately the speed of standard 3-methyl glucose in n-butanol:formic acid:water (100:23:77).

It was considered probable that the fast spots might be due to the presence of methyl esters. The syrup was, therefore, hydrolysed with sulphuric acid (5 ml.;1N) for 3 hours at 100°, neutralised with barium carbonate and examined paper chromatographically in butanol:benzene: pyridine:water (5:1:3:3) and butanol:ethanol:water (4:1:5) solvents. Xylose was present in quantity, with lesser quantities of glucose and glucurone. No rhamnose, 4-methyl glucose, or other methylated sugars were detected, the relevant standards being run on each paper. Some uronic acid of low R_G in the above solvents was also present. The faster sugars having R_G values 0.57 and 0.47 were not present after hydrolysis. Aniline oxalate solution was used as a developer.

Hydrolysis of Pure Barium Aldobiuronate.

A sample (circ. 100 mg.) of crude barium salt was separated into its components by chromatography on a wide, Whatman 3MM paper eluted with n-butanol:ethanol: water:ammonia (40:10:50:1). The uronic acid was removed from the dried paper in a Soxhlet extractor with water in the normal manner. Chromatographically pure aldobiuronic acid was obtained in this way.

The uronic acid was dried thoroughly at 40°/15 mm., and treated with methanolic hydrogen chloride (40 ml.;5%) under reflux for 24 hours at 60-70°. Acid was removed with silver carbonate, and the filtered solution,

evaporated to dryness at 40°/15 mm., was hydrolysed with hydrochloric acid (40 ml.;1.8N) for 18 hours at 100°. The acid was neutralised in the normal manner, and the product was examined paper chromatographically in various solvent mixtures. Xylose was heavily predominant, being present in much heavier concentration than the combined total of all other sugars.

Solvent.	Xylose.	Glucurone.	Glucuronic acid.	4-methyl Glucuronic acid.
n-Butanol:ethanol: water:acetic acid (40:10:50:1).	✓	✓	?	Covered by xylose.
Ethyl acetate: acetic acid:water (3:1:3).	✓	?	✓	✓
Ethyl acetate:acetic acid:formic acid: water (18:3:1:4).	✓	Heavy xylose spot obscures other sugars.		
n-Butanol:formic acid:water (100:23:77).	✓	✓	✓	✓

Some unhydrolysed aldobiuronic acid was also present. The quantities of glucurone and glucuronic acid present were greatly in excess of the 4-methyl glucuronic acid. Relevant standards were run.

Reduction of Pure Barium Aldobiuronate (through the Uronoside methyl ester.)

(a) Using lithium aluminium hydride.

A further quantity (circ. 100 mg.) of crude barium uronate was obtained free from xylose by separation on a filter sheet as described previously (see p.127).

The dry uronic acid obtained was treated with

methanolic hydrogen chloride (55 ml.;1½%) under reflux for 5 hours, neutralised with silver carbonate and evaporated to a glass at 40°/15 mm. This was dissolved in dry dioxan (10 ml.) and lithium aluminium hydride (200 mg.) was added at 80°, under reflux, for 7 hours. The solution was adjusted to pH 5 with sulphuric acid (2N), filtered, and reduced at 15 mm. to ¼ volume. To the mixture, water (20 ml.) was added and the whole was reduced again to ¼ volume before being hydrolysed with sulphuric acid (25 ml.;0.5N;100°) for 5-6 hours.

The acid was neutralised with barium carbonate, and the filtrate, after treatment, successively, with Amberlite resins IR 120(H) and IR 4B(OH) was examined paper chromatographically in n-butanol:ethanol:water: ammonia (40:10:50:1) and n-butanol:formic acid:water (100:23:77). Xylose (R_G, 0.15) and 4-methyl glucose (R_G, 0.22) were identified against standard samples. No glucose was observed. A quantity of unreduced aldobiuronic acid was also present.

(b) Using sodium borohydride, (181).

Crude barium aldobiuronate (circ. 150 mg.) was purified as before (see p.127) by separation on filter sheet, and the pure barium aldobiuronate obtained was converted to the ester glycoside (see above). This was dissolved in water (10 ml.) and sodium borohydride (100 mg.) was added. The mixture was shaken for 16 hrs. at room temperature, after which time glacial acetic acid was added to pH 4, the mixture filtered, made 1N with respect to hydrochloric acid, and heated under reflux at 100° for 6 hours.

Neutralisation and removal of silver ions was effected in the usual way, and traces of inorganic ions were removed by repeated filtration of the mixture after concentration to small volume at 15 mm., and by treatment with a mixed bed of Amberlite IR 120(H) and IR 4B(OH) resins.

The solution was evaporated at 40°/15 mm. to small volume and examined paper chromatographically in n-butanol:ethanol:water (4:1:5). Sugars corresponding in colour and R_G value to 4-methyl glucose (0.22) and xylose (0.15) standards were observed in approximately 1:1 ratio. A negligible trace of glucose, just observable on the chromatogram, was seen. No other sugars or uronic acids were present.

The mixture of sugars obtained was separated on a number of Whatman No.1 filter sheets in the usual manner, by elution with n-butanol:ethanol:water (4:1:5). The monomethyl glucose was extracted from the paper with water in a Soxhlet extractor and, after filtration through charcoal and "filter-cel", reduced to a syrup at 40°/15 mm.

Identification of the Monomethyl glucose fraction.

(a) Attempted preparation of monomethyl glucosazone.

The syrupy monomethyl glucose obtained from the separation above was mixed with water (1 ml.), hydrated sodium acetate (300 mg.), phenylhydrazine hydrochloride (125 mg.), and a trace of sodium bisulphite and heated in a small tube at 100° for 3¼ hours. The oil was filtered from the solution, washed with water, dissolved

in the minimum of hot ethanol, and recrystallised from aqueous ethanol. A yellow, flocculent precipitate which formed on cooling yielded minute dark brown crystals, m.p. 90-100° after drying. On standing at 0° for 72 hrs., a little oil separated from the filtrate. The oil was washed after removal, as above. It failed to crystallise from aqueous ethanol, from benzene, or after long standing at 0°.

(b) Attempted preparation of monomethyl glucosotriazole, (196).

A sample (circ. 10 mg.) of the dark brown crystalline osazone formed above was treated with copper sulphate solution (20 mg.;1 ml.) in a small tube at 100° for 2 hours. No crystals were obtained on cooling.

Reduction of Pure Aldobiuronoside methyl ester, (Large scale).

Crude barium aldobiuronate (2.3 g.) was converted into the aldobiuronoside methyl ester by treatment with methanolic hydrogen chloride (60 ml.;2%) for 5 hours at 60° and subsequent neutralisation. After evaporation at 15 mm. to a syrup, the product was reduced with sodium borohydride as described previously (p.129).

The mixture, after treatment with acetic acid, was made 1.5N with respect to hydrochloric acid, and heated on the boiling water bath for 6 hours. Neutralisation was effected with silver carbonate, and silver ions were removed with hydrogen sulphide. The total volume was reduced at 40°/15 mm. to 5 ml., and the liquid was filtered free from a heavy, white

crystalline residue. The residue was washed quickly with acetone (5 ml.;Analar) in which it was but slightly soluble, and the washings were added to the filtrate.

The filtrate, on evaporation at 40°/15 mm., yielded a heavy brown syrup which was dissolved in a little water (10 ml.) and treated with mixed Amberlite resins IR 120(H) and IR 4B(OH) to remove traces of inorganic material. Water was then removed at 40°/15 mm., to yield a clear, brown syrup (ca. 300 mg.).
(The low yield of syrup obtained above was due to sugar being retained on the white, crystalline residue. This residue could not be washed efficiently due to its solubility. In solution, it is not easily removed by resins, even when these are applied as a mixed bed.)

Residue.

The white crystalline residue (above), after drying, had m.p. > 210° with considerable blackening - the latter probably being due to syrup retained on the crystals.

An aqueous solution of the white crystals gave negative tests for silver with dilute hydrochloric acid, with hydrogen sulphide, and with para dimethylamino-benzylidene rhodanine in acetone, (219). Borate was shown to be present by a positive reaction with turmeric paper and pronounced colouration with para-nitrobenzene-azo-chromotropic acid.

The white crystalline residue, with adhering syrup, was therefore dissolved in hydrochloric acid (30 ml.;1N) and methanol (2 ml.), and heated under reflux on the boiling water bath for 5 hours, in an attempt to

volatilise borate present as methyl borate. This procedure was found to be ineffective. After neutralisation of the mixture in the usual way, and concentration to small volume, the solid white residue reappeared in unchanged amount.

Syrup.

The syrup, on chromatographic examination, showed 4-methyl glucose and xylose in approximately equal amounts, together with a trace of a sugar having the colour and R_G value of glucose, (0.08-0.09;developer, aniline oxalate).

(a) Separation and Identification of 4-methyl D glucose.

A quantity (circa. 200 mg.) of the syrup obtained above was separated on a number (3) of thick filter sheets (Whatman 3MM) using n-butanol:ethanol:water (4:1:5) solvent, and the monomethyl glucose was extracted from the paper strips in a Soxhlet extractor with water.

A sample (50 mg.) of the chromatographically pure monomethyl glucose so obtained was taken, together with phenylhydrazine hydrochloride (250 mg.), water (2 ml.), hydrated sodium acetate (700 mg.) and a trace of sodium bisulphite with which to form the osazone, (see p.130). After 10 minutes heating at 100°, a heavy, yellow crystalline precipitate appeared. After cooling, this was removed by filtration, washed thoroughly with water, dried, and recrystallised from hot benzene.

The filtrate was reheated at 95° on a water bath for a further 45 minutes, and a second crop of identical crystals was obtained. These were treated as described above.

The yellow needles obtained after recrystallisation had m.p. 153-4° undepressed by admixture with authentic 4-methyl D glucosazone prepared from synthentic 4-methyl D fructose ($_{215}$). The m.p. of a mixture of authentic 3-methyl D glucosazone with the crystals obtained above, however, showed marked depression below the m.p. for 3-methyl D glucosazone.

An X-ray powder photograph of the crystals (by the kindness of Dr.C.A.Beavers) gave a diagram identical with that obtained under the same conditions from standard 4-methyl D glucosazone, and different from that obtained from standard 3-methyl D glucosazone.

The crystals obtained above were thus of 4-methyl D glucosazone.

(b) Separation of the trace of glucose.

The filter strips bearing the sugar of R_G 0.08-0.09 from the above paper separation of the syrup (p.133) were extracted in a Soxhlet extractor with water, and the solution obtained was concentrated to small volume (I). Chromatographic examination of the solution (I) so obtained showed the sugar having R_G 0.08-0.09 to be present, with a trace of xylose. The sugar (R_G 0.08-0.09) appeared in repeated chromatograms to travel slightly behind standard glucose in n-butanol:ethanol:water (4:1:5) solvent.

To hydrolyse a possible disaccharide, the solution was made 1N with respect to hydrochloric acid, and boiled under reflux on a water bath at 100° (5 hrs.). After

neutralisation with silver carbonate, treatment with hydrogen sulphide and filtration, the product (II) was examined paper chromatographically. No change was detected in the composition of the mixture after hydrolysis. The sugar having R_G 0.08-0.09 travelled in the same position relative to standard glucose in n-butanol:ethanol: water (4:1:5), n-butanol:benzene:pyridine:water (5:1:3:3) and n-butanol:ethanol:water:acetic acid (40:10:50:1) solvents. For this feason it was considered unlikely to be a borate complex, or uronic acid.

The product (II) was examined ionophoretically under the conditions described on p.122 for 3 hours. Standard glucose, xylose, and arabinose samples were run. Spots corresponding to glucose, and xylose only were observed, and no sugars of lower mobility than glucose could be detected (M_G 0.85). Disaccharides hitherto examined (220) have shown extremely low mobilities under similar conditions of pH and potential difference.

The sugar of R_G 0.08-0.09 originally present in solution I was therefore taken to be glucose.

Viscosity Determinations.

(a) Methylated hemicellulose A, fraction 5.

The viscosity of a sample of methylated hemicellulose A (fraction 5) was obtained in m.cresol in an Ostwald viscometer (see p.62) at 20°. Using a concentration of 0.0705 g.mol. of repeating unit per litre, and a K_m of 12×10^{-4} (174), a molecular weight of 8960 was obtained, assuming the molecule to be of "ideal thread" form, and

assuming also that the above K_m value - derived for methylated cellulose in m.cresol - is applicable. This corresponds to a D.P. of 50 xylose residues. For a branched molecule, the figure quoted for D.P. may be expected to be lower than the correct value.

(b) Acetylated hemicellulose A.

Using a concentration of 7.125 g./litre in chloroform, the same conditions as above, and a K_m of 5.3×10^{-4} (174), a D.P. of 364 was obtained. Using K_m, 11×10^{-4} (186), a D.P. of 175 xylose residues was obtained. The constants used are those for cellulose triacetate in chloroform solution.

Reducing Power.

(a) Colorimetric method.

The method described on p.55, and the standard xylose curve, p.56, was used. A value for D.P. of 197 xylose residues was obtained using 4 cells in the determination, and 167 xylose residues using 2 cells.

(b) Alkaline hypoiodite.

The method described on p. 54 was used. Values of 130-144 xylose residues were obtained for the degree of polymerisation.

Determination of Molecular Weight by Isothermal Distillation (216).

The M.W. of methylated hemicellulose A (fraction 5) was determined by isothermal distillation of a solution in benzene (0.028 g. in 4.025 g. benzene) at $22.5 \pm 0.01^{\circ}$ by courtesy of Mrs. H. Zinkiewicz B.A. A value of 11,100 $\pm$ 500 was obtained, corresponding to a D.P. of 66-72 xylose residues.

DETERMINATION OF THE EFFECT OF THE DELIGNIFYING AGENTS USED ON PEAR CELL XYLAN.

Possible oxidation, and degradation.

The colorimetric, and alkaline hypoiodite methods for the estimation of reducing power (already described) were employed below. The object of the experiments was to measure the change in the reducing power suffered by a xylan of known chain length under the conditions obtaining on delignification. The xylan chosen was that from pear cell wall, (141).

Samples (0.5 g.) of pear xylan were treated with water (75 ml.), glacial acetic acid (5 ml.), sodium chlorite (5 g.), and sodium acetate (0.2 g.) at 30° for 20 hours, (A). No preliminary heating was given. Blank samples (B) were also prepared containing water (75 ml.), sodium acetate (0.2 g.), glacial acetic acid (5 ml.), and xylan (0.5 g.), to act as a check on the subsequent washing procedure.

After 20 hours at 30°, both sets of samples were washed (x 3) with water, alcohol, and ether, and air-dried, and the reducing power was determined in comparison with the reducing power of samples of untouched pear cell xylan.

(a) Colorimetric method, (169).

Polysaccharide.	Weight.	Mean log $\frac{I_o}{I}$	Equiv.wt.of xylose residues.	D.P.
Sample A.	82.52 mg.	0.024	0.334 mg.	247
"	98.60 mg.	0.046	0.392 mg.	250
"	76.44 mg.	0.024	0.334 mg.	231
Untouched sample	96.60 mg.	0.333	1.029 mg.	94
"	82.60 mg.	0.258	0.890 mg.	93

Polysaccharide.	Weight.	Mean log $\frac{I_o}{I}$	Equiv.wt.of xylose residues.	D.P.
Sample B	52.28 mg.	0.086	0.485 mg.	108
"	92.93 mg.	0.228	0.827 mg.	112
"	102.62 mg.	0.321	1.062 mg.	97

(b) Alkaline hypoiodite method, (see p.54). (D's.P. obtained).

Sample A, 153, 232.

Untouched sample, 49, 51.

Sample B, 210, 214, 206.

Of the three samples, the untouched samples alone appeared green on adding iodine solution (0.1N), probably due to residual copper from the copper complex purification (141).

DISCUSSION.

WHEAT STRAW HEMICELLULOSE.

The wheat straw (Var. White Victor) was cleaned and defatted with benzene and methanol, and lignin was removed by the method of Wise (12) using sodium chlorite-acetic acid mixture buffered to pH 4. This method has been shown to cause no appreciable degradation of hemicellulose so long as the delignification is not carried to absolute completion.

Following a suggestion by W.A. Bell (191) and from other evidence (190, 194), control experiments were undertaken to test the effect of the delignifying mixture on unprotected hemicellulose reducing "end" groups. It was found that when hemicellulose was exposed to acid chlorite under the conditions obtaining on delignification, some loss of reducing power as measured by alkaline hypo-iodite (97), and by Meyer's colorimetric method (169), was apparent. By analogy with the behaviour of simple sugars (192, 195), and oxycelluloses (193), it has been suggested that this is due to oxidation of the reducing "end" group to a carboxyl group by the acid chlorite mixture. However, the effect of the delignifying mixture on hemicellulose co-existing with lignin in natural tissues is not so certain. There is much evidence in favour of the contention that in these cases no such oxidation of "end" groups takes place. The evidence includes the complete absence of methylated aldonic acids from the products of hydrolysis of the methylated polysaccharides, and the approximate agreement

between estimates of chain length of various polysaccharides by the classical methylation-hydrolysis procedure, and by reducing group methods. The co-existence of lignin with hemicellulose in the tissues during delignification appears to protect the reducing "end" group of the hemicellulose from oxidative attack by the delignifying reagents. This may be due to the presence of a hemicellulose-lignin link through the reducing group of the hemicellulose to some group in the lignin, or to steric factors.

Under the best conditions, reducing group estimations of polysaccharide chain length give results of doubtful reliability. With alkaline hypoiodite (97) this is due to the overoxidation which is difficult to control, and with Meyer, Noelting and Bernfeld's method to the absence, for all polysaccharides except glucosans, of a disaccharide standard. In the present investigation, little weight has been placed upon information obtained by reducing group estimations; the structures proposed rest broadly on the evidence gained from methylation and hydrolysis procedures.

Crude xylan was extracted from the delignified straw with cold 4% sodium hydroxide solution and precipitated by acidification, and addition to ethanol. The crude polysaccharide obtained had 6.2% arabinose calculated on the basis of 100% total pentose. Arabinose and xylose were found to be the only sugar residues present apart from a small amount of uronic acid.

Purification.

The polysaccharide was purified by formation of the copper complex with Fehling's solution (88, 89, 141). Attempts to remove arabinose completely by this method, as was accomplished for xylans from esparto grass and pear cell wall (140, 141) were unsuccessful. After one purification treatment, the arabinose content was reduced to 5.4% (calculated on 100% total pentose basis), but this could not be reduced further by further complexing treatments. Lignin contents of the crude xylan, and the once purified sample, were not obtained. The apparent purification, represented by the figures for arabinose content on 100% total pentose basis, may therefore be too high, due to the concurrent reduction of residual lignin on purification through the copper complex. A more accurate indication of the purification from arabinose effected during the first complexing treatment may be gained by expressing the pentoses present in simple ratio: xylose/arabinose, crude:15.1/1.0, once complexed sample:17.5/1.0.

It was found that samples of the gelatinous copper complex which were allowed to stand for periods of up to 48 hours in 0.5N hydrochloric acid before precipitation in ethanol suffered a marked loss of arabinose. This, however, was felt to be an inadmissable method of purification in view of the risk of partial hydrolysis, although it has been reported that a galacto-araban which had been allowed to stand in 1N hydrochloric acid for 24 hours at room temperature showed no measurable loss of

arabinose. An araban, after 3 weeks under these conditions suffered a loss of 1½% (187). Assuming that the partial hydrolysis of a supposed arabo-xylan were to require much the same conditions as that for a galacto-araban, it is improbable that the observed loss of arabinose would be due to degradation.

Although care was taken to prevent the copper complex from remaining for an unnecessarily long time under acid conditions, it was found that, on adding dilute hydrochloric acid to decompose a suspension of the copper complex in water, decomposition was not complete until the mixture had been allowed to stand, in the cold, for at least ¼ hour. Acetic acid was found to be of little use, giving a blue, colloidal solution which was heavily buffered. If alcohol were added immediately the suspension of copper complex had been dissolved in the hydrochloric acid, then there was precipitated a mixture of white polysaccharide and blue, undecomposed copper complex. This solid mixture, when dry, was only partially soluble in dilute sodium hydroxide solution.

An attempt was made, on this basis, to fractionate the hemicellulose on the probability that the different polysaccharides present might yield copper complexes of varying stability. This method proved to be unsuccessful, the arabinose:xylose ratio remaining the same in each fraction.

The polysaccharide was successfully purified by fractional solubility in hot aqueous ethanol, the residue after this treatment being found to have a lowered

arabinose content. This method has been used to give a partial separation of an arabo-xylan from xylan in esparto grass (67). After two such treatments, the arabinose present was reduced to 0.6% estimated by periodate after separation of the hydrolysis mixture on paper chromatograms. Using rhamnose as a reference sugar, and correcting for loss of xylose on hydrolysis, 87.7% of the purified polysaccharide was accounted for as xylose. A uronic anhydride determination by the method of Svenson et al (167) gave a mean value for CO_2 of 0.79%. Thus, with 0.7% ash, and allowing for 6.5% of the polysaccharide to be present in the hydrolysis mixture as aldobiuronic acid, 95.5% of the total polysaccharide was accounted for. Lignin values varying from 0.4% (166) to 1.5% (165) according to the method of determination adopted, raised this figure to approximately 96.5%.

The modified method of lignin estimation due to Ritter, Seborg and Mitchell (166) gave the lower of the two values obtained for lignin content. This is to be expected since the reduction, by Ritter et al, of the time of contact of the material with 72% sulphuric acid from 16 hours to 2 hours, at a temperature not exceeding 20°, is claimed to reduce the positive error due to condensation of lignin residues with furfural. It has been shown that the presence of furfuraldehyde causes a marked increase in the amount of apparent lignin isolated (203). The value of 0.4% obtained for the lignin content of the purified polysaccharide may,

therefore, be taken to be the more correct value.

The purified xylan had $[\alpha]_D^{20}$ - 93° (c,0.21 in 4% sodium hydroxide solution), and OMe, 0.4%.

Periodate Oxidation.

The use of periodic acid to reveal the existence, in a compound, of hydroxyl groups on contiguous carbon atoms is now general practice (213, 214). It has been shown that in a compound having hydroxyl groups on two adjacent carbon atoms, oxidation with periodic acid may take place as follows:-

$$R{\cdot}CH(OH){\cdot}CH(OH){\cdot}R' + HIO_4 = RCHO + R'CHO + HIO_3 + H_2O$$

The reaction is normally quantitative, and one mole of periodate is consumed for each C-C bond broken. The periodate so consumed may be determined quantitatively (168). Should hydroxyl groups on non contiguous carbon atoms only be present, no oxidation occurs.

When more than two adjacent carbon atoms carry hydroxyl groups, oxidation may proceed further with the production of formic acid from -CH(OH)·CH(OH)·CH(OH)- groups and formaldehyde from -CH(OH)·CH_2OH groups. The formic acid and formaldehyde liberated may be determined quantitatively, and this affords a ready method for the assay of these groupings in the molecule.

The pure xylan (Xylan F), when subjected to oxidation by the periodate ion under the usual conditions, released a quantity of formic acid which did not reach a constant value. Such behaviour has been reported previously for polyuronide materials (141). It is

therefore, difficult to attach precise significance to values of chain length obtained from the results. By extrapolating the curve to zero time, a value 6.7 to 6.8 ($\times 10^{-2}$) moles formic acid released per $C_5H_8O_4$ unit is obtained. This corresponds to a D.P. for the polysaccharide of 44-5 residues (calculated as xylose residues), assuming an unbranched chain, with the uronic acid residues contributing no formic acid (apart from the effect of slow overoxidation). If the assumption is made that the uronic acid residues present do each contribute one mole of formic acid, the value obtained corresponds to a D.P. of 59-60 xylose residues. Comparing these figures with results obtained from isothermal distillation, and methylation data, the periodate oxidation evidence alone would appear to favour the existence, in the molecule, of methylated uronic acid residues.

The periodate uptake, which reached a constant value of 0.87 moles periodate consumed per $C_5H_8O_4$ unit, indicated a 1:4 link between the xylose residues in the chain. This mode of linking was confirmed by methylation data (below). The somewhat low value obtained for the periodate uptake is, however, difficult to explain.

Hydrolysis of the oxidised material, and paper chromatographic examination revealed a trace of xylose, possibly having origin in branch points immune to attack by the periodate ion.

This behaviour - of a constant periodate uptake with a steady increase in formic acid released - was repeated with hemicellulose A from beechwood (Part II).

Methylation.

The polysaccharide was methylated 10 times with dimethyl sulphate and sodium hydroxide under nitrogen, and the product was fractionated with various mixtures of chloroform-light petroleum. Fractions having methoxyl contents of 32.9% and 34.0% were obtained. The former fraction was treated a further 4 times with dimethyl sulphate and sodium hydroxide before being methylated with methyl iodide and silver oxide. The latter fraction was afforded two methylations with methyl iodide and silver oxide and both products were combined to give a crude, methylated xylan (I) having OMe, 38.0%. A second fractionation gave, in very good yield, a pure methylated xylan (B5) as a cream-white powder having OMe, 38.2%, $[\alpha]_D^{19}$ -82.7° (c, 0.45 in chloroform).

The fully methylated xylan (I) was completely soluble in a mixture of hot chloroform:light petroleum b.p. 60-80 (30:70). The pure, fully methylated xylan (B5) which comprised 75% of the crude, fully methylated xylan (I) at this stage, was soluble in boiling chloroform: light petroleum b.p. 60-80° (30:70), but insoluble in a boiling mixture of the same solvents in the ratio 25:75. This rather precise solubility distinction would appear to be common to all xylans so far examined, including those with combined uronic anhydride residues, and those without. Thus esparto (140), pear cell wall (141), beechwood (see later), oat straw (204) and barley straw (205) xylans behave in this manner.

Viscometric measurements were made on the fully methylated xylan (B5) in m.cresol at 20± 0.1° using an Ostwald viscometer. Staudinger constants (174) for methylated cellulose in m.cresol were applied to the results, and a M.W. of 4,700 was obtained for the methylated polysaccharide. This corresponds to an average D.P. of 26-27 xylose residues. The assumption is made that the molecule is "ideal", i.e. unbranched, and that the Staudinger constant used is applicable. Since the molecule is not "ideal" - (it possesses a dependent glucuronic acid residue) - the true value may be expected to be higher than indicated.

Hydrolysis of the Methylated Xylan.

The fully methylated xylan (B5), on hydrolysis and paper chromatographic examination yielded sugars corresponding to trimethyl pentose (R_G, 0.94), 2:3-dimethyl xylose (R_G, 0.74) and monomethyl xylose (R_G, 0.38). The molar ratio of these pentose derivatives was estimated using buffered alkaline hypoiodite, after separation of the individual sugars on paper chromatograms. Trimethyl pentose, later identified as 2:3:4-trimethyl D xylose amounting to 2.8 to 3.3 mol. percent of the total pentose present, was obtained. This corresponds to a molecule having one xylose non-reducing "end" group per 30 to 36 xylose residues, the uronic acid residues not being taken into consideration. The estimated quantity of monomethyl xylose was 2.9 to 3.4 molar percent, but some of this material may have had origin in undermethylation, or demethylation on hydrolysis (see also p.185).

The figures above, calculated on the basis of molar percent total free pentose do not take into account the 3% (approx.) of the total pentose bound to the uronic acid as aldobiuronic acid.

The fully methylated xylan was hydrolysed with methanolic hydrogen chloride until the rotation of the mixture became constant, and the resulting glycosides were hydrolysed with hydrchloric acid in the usual way. Difficulty was experienced at this point in effecting complete solution of the methanolysed mixture, solid being precipitated on adding hydrochloric acid for the final hydrolysis. Continued boiling under reflux failed to dissolve the solid, which was finally removed, dried, and afforded further methanolysis treatment. The resultant fully methanolysed mixture was combined with the liquid and hydrolysed with hydrochloric acid to constant rotation, acid being then removed on a column of Amberlite ion exchange resin IR 4B(OH).

The resultant syrup was separated on a column of cellulose eluted first with a mixture of n-butanol:light petroleum b.p. 100-120° (30:70) saturated with water and, after the trimethyl xylose and dimethyl xylose had all been collected, by n-butanol saturated with water. When the monomethyl xylose had all been collected, a water wash removed a small quantity of uronic acid from the column. The total yield of pure sugars, including uronic acid, from the column separation was 93.5%.

Fraction 1 (Trimethyl pentose).

This fraction, on paper chromatographic examination,

was shown to contain a trace of monomethyl xylose. The purified syrup (149 mg.) was therefore separated on washed, thick filter sheet , and syrup (144 mg.) was obtained which was chromatographically pure to an aniline oxalate spray, but failed to crystallise on storage. The syrup had a methoxyl content of 47.6%, compared with the required 48.4% for pure trimethyl pentose. Buffered alkaline hypoiodite oxidation, however, indicated only 78.9% pure aldopentose.

A sample of the syrup was hydrolysed, and examined paper chromatographically. Some (circ. 20%, visually) 2:3-dimethyl xylose was identified along with the trimethyl pentose in the hydrolysis products. This observation, with the result of the hypoiodite oxidation above, indicated that some 2:3-dimethyl methyl xyloside was present in the trimethyl pentose syrup.

The remaining trimethyl pentose syrup (133 mg.) was therefore rehydrolysed with dilute acid to constant rotation, and the resulting neutral syrupy product was separated on a semi-micro column of cellulose by elution with a mixture of n-butanol:light petroleum b.p. 100-120° (30:70) saturated with water. Pure trimethyl pentose (101.1 mg.) and pure 2:3-dimethyl xylose (25.2 mg.) were obtained, recovery on the column separation being 97%.

The pure trimethyl pentose crystallised completely on removing the solvent, yielding crude brown crystals m.p. 87-8° which, on re-crystallisation from dry ether had m.p. 89° undepressed by admixture with authentic 2:3:4-trimethyl D xylose. The crystals had the correct

rotation and methoxyl content for the above sugar, whilst oxidation with alkaline hypoiodite indicated 99.1% aldopentose.

The identity of the 2:3:4-trimethyl D xylose was confirmed by the preparation of the crystalline anilide.

The 2:3-dimethyl xylose obtained from the semi-micro column separation had a methoxyl content of 34.0%, and purity by buffered alkaline hypoiodite was 98.1%. The derived anilide, on recrystallisation from ethyl acetate-light petroleum gave colourless needles m.p. 122°, not depressed by admixture with authentic 2:3-dimethyl D xylose anilide recrystallised from the same solvent or by admixture with the anilide obtained from Fraction 2.

Fraction 2.

The dimethyl xylose (4.180 g.), obtained from the large column separation, crystallised slowly on seeding with authentic 2:3-dimethyl D xylose. The syrup had the correct methoxyl content and rotation for 2:3-dimethyl D xylose, and the crystals had m.p. 77-8°. On chromatographic examination, the fraction gave a single, discrete spot, R_G, 0.74.

From a sample of Fraction 2, the crystalline anilide was prepared. This appeared to be dimorphous having m.p. 123° on recrystallisation from ethyl acetate-light petroleum, and m.p. 134-5° after washing with dry ether and drying in air. Both melting points were undepressed on admixture of the sample with authentic 2:3-dimethyl D xylose anilide treated in the same manner.

A sample of Fraction 2 was oxidised with bromine water and heated to yield a syrupy lactone from which the crystalline amide was obtained. The crystals, recrystallised from ethyl acetate had m.p. 132°, undepressed by admixture with authentic 2:3-dimethyl D xylonamide.

The crystalline amide prepared failed to give a positive Weerman reaction.

Fraction 2 was therefore 2:3-dimethyl D xylose.

Fraction 3.

The syrup (139 mg.) gave crystals which, on recrystallisation from methanol, had m.p. 135-6°, undepressed by admixture with authentic 2-methyl D xylose. The fraction compared, paper chromatographically, both in colour and position with standard 2-methyl D xylose, but not with standard 3-methyl D xylose; it had methoxyl content 18.4%; $[\alpha]_D^{19}$ + 30° (c, 1.6 in water), and a purity by alkaline hypoiodite of 99.0%.

Fraction 3 was therefore 2-methyl D xylose. Confirmation was obtained by the preparation, melting point, and mixed melting point of the crystalline 2-methyl D xylose anilide.

Between fractions 3, and 4, a trace of xylose (< 5 mg.) was eluted from the cellulose column, and identified by paper chromatography against standard xylose. It had R_G, 0.15, and gave the pink colouration characteristic of pentoses on development with aniline oxalate solution.

Fraction 4.

Crude Fraction 4 (308 mg.) was removed from the

cellulose column with water. It gave, on chromatographic examination, two cherry red, heart shaped spots indicative of uronic acids. The faster, which was heavily predominant had R_G 0.09-0.10, identical with the uronic acid isolated by Chanda et al (141) from methylated pear cell xylan. The slower uronic acid had R_G, 0.05.

On demethylation of a sample of crude fraction 4, only xylose and xylose derivatives could be detected. The latter were 2-methyl xylose (R_G, 0.38), and a trace of 2:3-dimethyl xylose (R_G, 0.74) identified against standard samples.

A sample of crude fraction 4 was hydrolysed for 16 hours with sulphuric acid (1N;100°), and an insoluble, crystalline residue was obtained. This was shown, under a polarising microscope, to be calcium sulphate which had probably been carried over as calcium aldobiuronate from the final neutralisation of the hydrolysate of the methylated xylan before separation on the cellulose column. The hydrolysate of the crude fraction 4 was examined paper chromatographically and shown to contain some 2-methyl xylose, a trace of 2:3-dimethyl xylose together with some of the uronic acid originally present (R_G, 0.10), and a faster uronic acid (R_G, 0.16) travelling just ahead of standard xylose. The latter uronic acid was probably 2:3:4-trimethyl glucuronic acid, produced, with 2-methyl xylose, on hydrolysis of the linkage constituting the aldobiuronic acid. The trace of 2:3-dimethyl xylose was considered to have origin in a small quantity of oligosaccharide-uronic acid present in

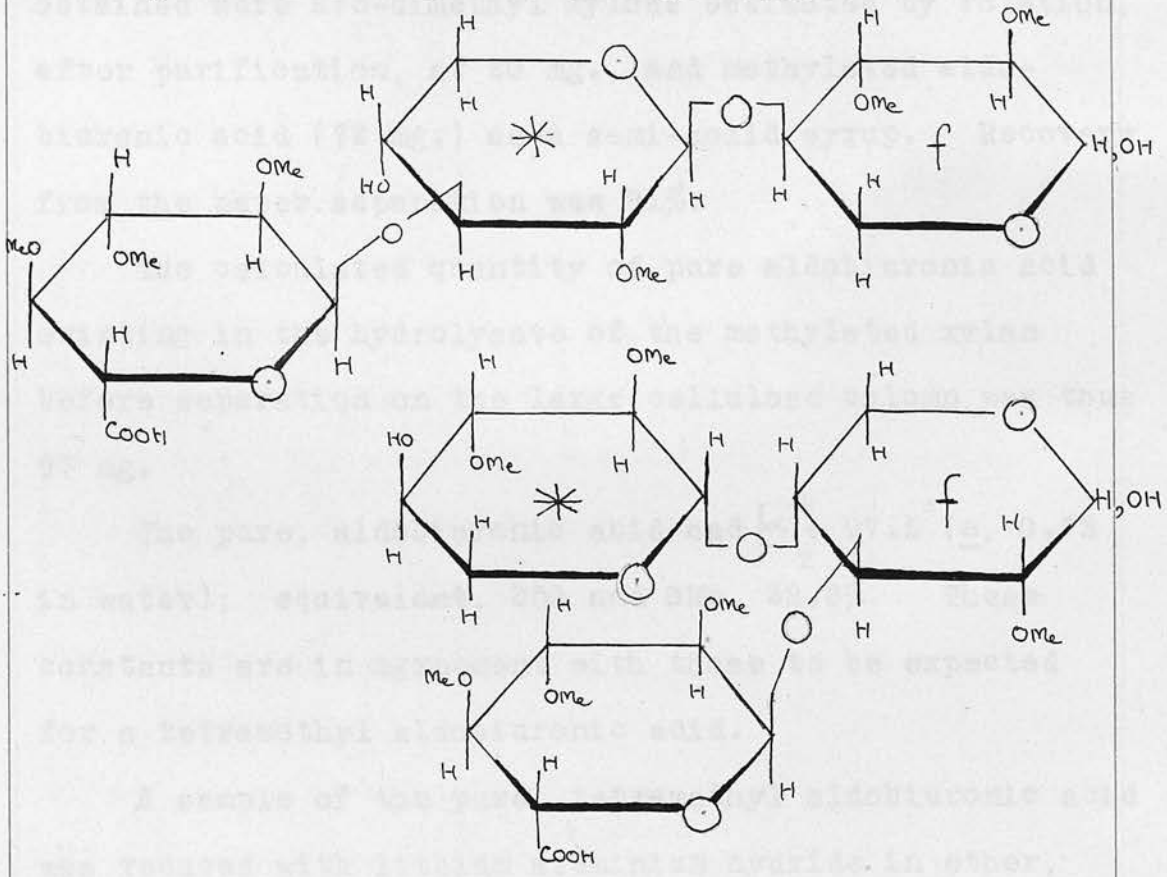
the fraction before hydrolysis.

A sample of crude fraction 4 was reduced with lithium aluminium hydride in ether, after formation of the glycoside esters, and the product was hydrolysed and examined paper chromatographically. By the colour of the spots produced on spraying the paper with aniline oxalate solution, and by the R_G values in comparison with standard sugars, there was identified in the hydrolysate 2:3:4-trimethyl glucose and 2-methyl xylose in approximately equal quantities, together with a trace of 2:3-dimethyl xylose.

Samples of crude fraction 4 were oxidised with sodium metaperiodate after separation on paper chromatograms (172). A spray of Schiff's reagent was used with which to detect formaldehyde. The uronic acid having R_G, 0.09-0.10 gave the magenta spot of a Schiff-aldehyde complex, whilst the uronic acid having R_G,0.05 gave no reaction. This was taken to indicate that the former only, of the two uronic acids, liberated formaldehyde on treatment with periodate, and that therefore only the former contained an unsubstituted $CH(OH)\cdot CH_2OH$ grouping.

If further xylose residues were attached to the aldobiuronic acid (example Figs. A or B), as would be the case if the sugar having R_G, 0.05 were an oligosaccharide-uronic acid, it follows that either the pyranose ring of the residue marked * cannot be opened and thus no formaldehyde is liberated on treatment with periodate, or the hydroxyl on C_4 in the residue marked

f is blocked, again with the same result. Larger oligosaccharide-uronic acid units than those shown in the diagrams will behave in similar manner.



Neither aldobiuronic acid no oligosaccharide-uronic acid may be expected to yield formic acid on treatment with periodate.

The formic acid released on treatment with periodate was tested for in a separate determination using a spray of potassium iodide solution. No formic acid was detected from either uronic acid, although a control determination on a glucose standard yielded an iodine stain - indicating formic acid- after similar treatment.

The remaining quantity of crude fraction 4 (270 mg.) was hydrolysed with dilute acid to decompose the small quantity of oligosaccharide-uronic acid present to aldobiuronic acid. After neutralisation, the aldo-

biuronic acid was separated from a little 2:3-dimethyl xylose on washed, thick filter sheet. The fractions obtained were 2:3-dimethyl xylose estimated by rotation, after purification, at 20 mg., and methylated aldobiuronic acid (72 mg.) as a semi-solid syrup. Recovery from the paper separation was 91%.

The calculated quantity of pure aldobiuronic acid existing in the hydrolysate of the methylated xylan before separation on the large cellulose column was thus 97 mg.

The pure, aldobiuronic acid had $[\alpha]_D^{16}$ + 97.5° (c, 0.72 in water); equivalent, 350 and OMe, 32.2%. These constants are in agreement with those to be expected for a tetramethyl aldobiuronic acid.

A sample of the pure, tetramethyl aldobiuronic acid was reduced with lithium aluminium hydride in ether, as described previously, and examined paper chromatographically. Approximately equal quantities of 2:3:4-trimethyl glucose and 2-methyl xylose only, were observed. The mixture was separated on a thick, filter sheet, and the component sugars were estimated with buffered alkaline hypoiodite. A proportionality of 1.0:1.1 mol. was obtained for 2:3:4-trimethyl glucose: 2-methyl xylose.

A sample of the sodium salt of the pure, methylated aldobiuronic acid was subjected to oxidation in the dark by the periodate ion, and the uptake of periodate was determined using excess of arsenite solution followed by back titration with standard iodine solution (168).

A value, after 26 hours, of 0.8 mol. periodate consumed per $C_{15}H_{26}O_{11}$ unit was obtained.

The yields of the various sugars are given in the table below, together with the weights, corrected for column and paper losses, calculated to have been in the hydrolysed, methylated mixture before separation.

The amount of 2:3:4-trimethyl D xylose obtained corresponds to one xylose "end" group per 43-44 other residues. The amount of 2:3:4-trimethyl D xylose obtained is also given calculated as a percentage of the total pentose, for the purposes of comparison with the hypoiodite estimations.

The amount of aldobiuronic acid obtained corresponds to one aldobiuronic acid unit per 107-8 other residues. This is considerably lower than the 3% (mol.) aldobiuronic acid to be expected from the uronic acid determinations on the unmethylated polysaccharide. The discrepancy may be due to loss of uronic acid on the resin used for neutralisation of the hydrolysate of the methylated xylan. An attempt was made, unsuccessfully, to reclaim this uronic acid bound to the resin, by elution with mineral acid.

Cellulose column separation.

	Final yield of pure material.	Corrected wt. present in hydrolysis mixture.	Mol.% (total polysacc.)	Mol.% (total pentose)
2:3:4-trimethyl D xylose.	0.101 g.	0.120 g.	2.30%	2.32%
2:3-dimethyl D xylose.	4.180 g.	4.522 g.	93.46%	94.30%
2-methyl D xylose.	0.139 g.	0.148 g.	3.32%	3.35%
2-methyl 3-(2:3:4-trimethyl D glucuronosido) D xylose.	0.072 g.	0.097 g.	0.93%	-

Hypoiodite determinations.

Sugars present in the hydrolysate of the methylated xylan.

	Molar %, total pentose. Detn. 1.	Detn. 2.
2:3:4-trimethyl D xylose.	3.3% (0.74 mg.)	2.8% (0.56 mg.)
2:3-dimethyl D xylose.	93.5% (6.50 mg.)	94.3% (5.80 mg.)
2-methyl D xylose	3.4% (0.65 mg.)	2.9% (0.49 mg.)

The figures in brackets are the calculated weights of oxidised material.

Weights quoted for these sugars are x 3. (see Exptl.)

Examination of the Acidic Fraction of Straw Xylan.

A quantity of pure xylan (xylan F) was hydrolysed under conditions calculated to yield most of the uronic acid as aldobiuronic acid. An attempt was made to separate the aldobiuronic acid by adsorbtion of it on Amberlite IR 4B(OH) ion exchange resin from acid solution,

and cause its subsequent removal by elution of the resin with mineral acid and water (187). Much difficulty was, however, experienced in carrying this into effect. Most of the uronic acid failed to be adsorbed on the resin, probably due to the large size of the uronic acid molecule.

Separation of the aldobiuronic acid from free xylose in the hydrolysate fractions was then attempted by precipitation of the former as the barium salt in alcohol. This process was hindered by the small quantity of uronic acid present, and by the tendency for xylose to be co-precipitated. A small amount of solid was finally obtained which, was very soluble in water and whose solution gave a white precipitate of barium sulphate on the addition of dilute sulphuric acid. On chromatographic examination of the solid, the barium salt of an oligo-saccharide-uronic acid was shown to be present, together with a little free xylose. Hydrolysis of this barium salt gave glucurone, glucuronic acid and xylose. No trace of 4-methyl glucuronic acid was detected, the hydrolysate being examined against the relevant standards in three solvent systems. From this fact, and from the methoxyl content of the unmethylated polysaccharide (0.4%), which was sufficiently low to be accounted for by the residual lignin present, the uronic acid residues present in the free polysaccharide were considered to be unmethylated.

From parallel experiments on the acidic fraction of

beech hemicellulose A, it may be concluded that although extensive demethylation of methyl uronic acid residues does take place on vigorous hydrolysis of methyl aldobiuronic acid units, some methyl glucuronic acid may nevertheless be identified in the hydrolysis products, even after hydrolysis under the most vigorous conditions. The complete absence of methyl glucuronic acid in the case of the straw xylan hydrolysate would therefore lead to the conclusion that no methyl aldobiuronic acid was originally present in the acidic fraction from this source.

In a recent paper by Bishop (202), published shortly after the work described herein was completed, the isolation of an aldobiuronic acid from wheat straw holocellulose is reported. The acid obtained by Bishop was shown to be 3-(xylopyranosyl)-α-D glucopyranoside by a methylation and hydrolysis procedure. During the course of the above work by Bishop, the β isomer of this aldobiuronic acid was synthesised. Hydrolysis of the wheat straw polysaccharide and precipitation of the acidic fraction as the barium salt in methanol gave Bishop an oligosaccharide-uronic acid barium salt (Barium salt "A") in which the molar ratio methoxyl/uronic anhydride was 1.1/1.0. On hydrolysis of the "Barium salt A", and precipitation of the neutral product in ethanol, the barium salt of an aldobiuronic acid (Barium salt "B") was obtained having the molar ratio methoxyl/uronic anhydride , 0.35/1.0. These findings would appear to show that the uronic acid residue exists in the poly-

saccharide carrying a single methoxyl grouping. Hydrolysis of the oligosaccharide-uronic acid residue under fairly vigorous conditions (5% sulphuric acid; 97°; 12 hrs.) would then be assumed to have caused extensive demethylation resulting in the figures quoted.

Whilst this is a possible explanation, it must be remembered that the barium salts "A" and "B" were precipitated in methanol and ethanol respectively, and these solvents are notoriously difficult to remove by simple drying, even at high temperatures. No special precautions appear to have been taken to remove occluded alcohol from the barium salt samples before analysis for methoxyl. Quoted molar ratios for methoxyl/uronic anhydride may therefore prove to be misleading.

More significant evidence for the existence of an aldobiuronic acid bearing a single methoxyl group, in wheat straw hemicellulose, exists in the work of Adams (98), and Adams and Castagne (130). Methoxyl determinations giving a ratio for methoxyl/uronic anhydride of approximately unity were obtained (130) on hemicellulose materials from wheat straw which had been fractionated with various mixtures of chloroform and light petroleum. In this case, the possibility that occluded solvent might give rise to an apparently high methoxyl content cannot arise. The hemicellulose examined by these workers differs, however, from that which forms the subject of the present investigation in that it (a) contained a much higher percentage of arabinose and uronic anhydride (13.6%, and 10.6%,

respectively) and (b) was water soluble.

Occluded alcohol as a cause of high methoxyl values in hemicellulose preparations might well be advanced to explain the unduly high figures obtained for this grouping by Adams and Castagne with various fractions of wheat straw hemicellulose (128). Fractions of this hemicellulose gave methoxyl/uronic anhydride ratios varying from 1.0/1.0 and 2.1/1.0 to 6.5/1.0 and 10.7/1.0, the latter being greatly in excess of any value which could be explained by the existence, in the fraction, of residual lignin. All the samples had been treated with alcohol, and some with alcohol followed by ether, before vacuum drying. Solvent exchange through ether is almost as inefficient a method of removing residual methanol or ethanol as is simple drying. It is worthy of note, however, that fractions yielding the highest methoxyl values were those dried directly from alcohol.

Acetylation of Xylan F.

From the pure xylan (Xylan F), the xylan diacetate was prepared by two acetylations, at 45°, using pyridine and acetic anhydride. Formamide was employed as a dispersing agent during the first acetylation (173). The product had theoretical acetyl content.

Reducing Power of Xylan F.

An approximate value for the molecular size of the xylan was sought by measurement of the reducing power of the polysaccharide using two different methods. The unreliability of values for D.P. based on reducing group methods has already been discussed (see p.140).

In the first method, the polysaccharide was oxidised in the dark with alkaline hypoiodite buffered at pH 11.4, and the consumption of iodine was measured after 5 and 16 hours. Results after 5 hours indicated that, after this time, oxidation of all potential aldehydic groups to carboxyl groups was still incomplete. After 16 hours, a value for D.P. corresponding to one reducing group per 164 other residues was obtained.

The second method used was that due to Meyer, Noelting and Bernfeld (169). This depends on the estimation, photometrically, of the highly coloured compound formed when 3:5-dinitro-salicylic acid is reduced in hot, alkaline solution by the polysaccharide. A standard curve, which may be constructed using known, similarly treated samples of xylose, was employed from which a value of 43 xylose residues was obtained for the D.P. of the polysaccharide. A more accurate value for D.P. of a polysaccharide is claimed for this method if a standard curve constructed for the corresponding biose is used, rather than the curve for the monosaccharide. Unfortunately, significant quantities of xylobiose were not available.

Since the reducing power per mole of disaccharide is greater than that of the corresponding monosaccharide, the value obtained for the D.P. may be considered to be slightly low due to this fact. The value obtained also takes no account of any effect the uronic acid residues present may have on the dinitro-salicylic acid reagent, the total weight of the polysaccharide being calculated as xylose residues. For these reasons, and for others

previously stated, little value can be placed on the figures calculated for molecular size of the polysaccharide by Meyer's method.

Isothermal distillation of Methylated Xylan, (216).

The M.W. of the fully methylated xylan (B5) was determined by isothermal distillation of a solution in benzene (0.0929 g. in 20 ml.) at 22.5° by courtesy of Dr. C.T. Greenwood. A value of 8,000± 500 was obtained, corresponding to a D.P. of 47-53 xylose residues.

Structure.

On the basis of the above results, the following is put forward as a probable structure for wheat straw xylan:-

An unbranched chain of approximately 43 D xylopyranose residues linked β 1:4 with a single, unmethylated glucuronic acid residue, in each chain, attached to position 3 of a xylopyranose residue in the main chain. Figure I illustrates this structure.

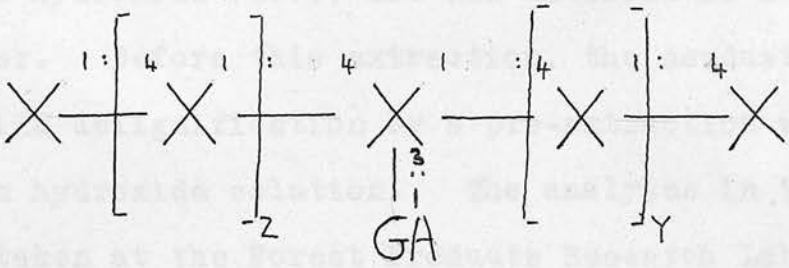


Fig. I.

Y+Z = 40 approx.

X = D xylopyranose.

GA = D glucuronic acid.

The monomethyl xylose present in the hydrolysate of the methylated xylan might be explained by undermethylation, and demethylation on hydrolysis. It may be noted, however, that there is a close correspondence between the amount of monomethyl xylose thus isolated, and the amount of uronic acid present in the polysaccharide, (2-methyl xylose, 3.3 mol.%; uronic anhydride, 3.2 mol.%). This correspondence is repeated in the results of hydrolysis of the methylated beechwood xylan, (Part II). On pp.185-6 a possible explanation is discussed.

BEECHWOOD HEMICELLULOSE A.

The beechwood hemicellulose A used in the investigation was kindly made available by Mr. I.R.C. McDonald of the Forest Products Research Laboratory at Princes Risborough. It was isolated from European beechwood (Fagus sylvatica) by extraction with dilute (1N) sodium hydroxide (179), and was received as a fine white powder. Before this extraction, the sawdust was afforded mild delignification by a pre-extraction with 0.1N sodium hydroxide solution. The analyses in Table I were undertaken at the Forest Products Research Laboratory.

Table I.

Methoxyl,	4.5%
Pentosan, (calculated as xylan, estimated as xylose, and uncorrected for uronic anhydride),	84.8%
Pentosan, (corrected for uronic anhydride),	81.4%
Uronic anhydride,	10.4%
Sulphuric acid lignin,	0.9%

Table I (contd.)

Acid soluble lignin,	1.0%
Total polysaccharide,	91.1%
Ash, as Na_2O,	1.56%
$[\alpha]_D$ (c, 0.4715 in N sodium hydroxide),	$[\alpha]_D$ -90.6°

The uronic anhydride content, total xylose, and rotation of the polysaccharide were re-determined by the author.

The rotation obtained was $[\alpha]_D^{20}$-89.4° (c, 0.347 in 4% sodium hydroxide).

Methoxyl determinations by the micro, volumetric method of Vieböch and Brecher (197, 198, 199) afforded a mean value of 1.67% methoxyl. A determination by the gravimetric method of Zeisel (199) however, gave a value of 4.9% methoxyl, in agreement with the value of 4.5% obtained at the Forest Products Laboratory where the methoxyl determination was also performed gravimetrically. The value obtained by the volumetric method was taken as correct, since the precipitate obtained in the gravimetric method did not appear to be wholly silver iodide.

The uronic anhydride content, determined by the method of Svenson, McCready and M'Lay (167) gave an average value of 9.5% uronic anhydride, (10.5% as uronic acid). This figure is in agreement with the figure of 11% glucuronic acid earlier quoted, for hemicellulose A from beechwood, by O'Dwyer (210), using a modified CO_2 method due to Nanji, Paton and Ling (211).

Using the figure of 1.67% methoxyl, the methoxyl/

uronic anhydride ratio obtained is thus exactly 1.0/1.0.

The polysaccharide, examined paper chromatographically after hydrolysis was shown to contain xylose, uronic acid and a trace of rhamnose. The rhamnose was not present as a free sugar impurity with the polysaccharide since the unhydrolysed hemicellulose gave no indication of rhamnose, paper chromatographically.

Xylose, estimated with periodate (175) after separation paper chromatographically from the hydrolysed polysaccharide, accounted for 81.6% of the total hemicellulose. Rhamnose, estimated colorimetrically using the arseno-molybdate Somogyi reagent (180, 185) was present in the polysaccharide to the extent of 0.40%, calculated as rhamnose hydrate. For the xylose and rhamnose determinations, galactose was employed as a standard reference sugar. The quantity of xylose present (81.6%) as estimated by periodate is in close agreement with the figure obtained for total pentose by furfural (81.4%), and also with the total percentage of methylated xylose derivatives later isolated from the cellulose column separation of the hydrolysed, methylated hemicellulose.

Attempts were made to purify the polysaccharide by formation of the copper complex, and by fractional solution in hot, aqueous ethanol as had been successfully accomplished for wheat straw xylan (Part I). No decrease in the rhamnose content was effected by these treatments.

Extraction of the hemicellulose A with cold 0.1N

sodium hydroxide solution was found to be equally unsuccessful, both the residue and the re-precipitated extract being chromatographically identical.

Purification by fractional precipitation appeared to be more promising. On acidifying a solution of the hemicellulose A in sodium hydroxide with acetic acid to pH 7, most of the hemicellulose (circ. 80%) was precipitated. A colloidal solution remained which gave no further precipitate on increasing acidity, but on addition to ethanol yielded a gelatinous solid (representing 13% of the total hemicellulose). On chromatographic examination of the hydrolysate of the latter solid, it appeared that xylose only was present, all rhamnose and uronic acid having been removed. However, a uronic acid determination by the CO_2 method (167) gave a figure of 9.8% uronic anhydride. The apparent purification from uronic acid effected was due to the presence of inorganic salts on the chromatogram papers. These caused a white patch, undeveloped by the aniline oxalate solution on development of the chromatogram, over the position near the starting line occupied by the uronic acid.

The gelatinous solid precipitated, (above, on addition of alcohol) probably consisted of shorter chain material present in the hemicellulose.

It was felt to be unlikely, both from the percentage of rhamnose originally present in the hemicellulose, and its complete removal - although in very low yield - by physical means, that rhamnose could exist in the hemicellulose as an integral part of the molecule. This

was later supported by the absence of rhamnose and rhamnose derivatives from the hydrolysis products of the methylated polysaccharide.

In the experiments described in the ensuing part of this investigation, the whole hemicellulose A, as supplied by the Forest Products Laboratory, was used unless otherwise stated.

Periodate Oxidation.

Beech hemicellulose A was oxidised by the periodate ion under the usual conditions. The uptake of periodate reached a constant value of 1.0 mol. periodate consumed/ $C_5H_8O_4$ unit after 164 hours. The formic acid released did not reach a constant value being 9.31×10^{-2} mol. $HCOOH/C_5H_8O_4$ unit after 284 hours, still increasing. This behaviour is usual for polysaccharides containing combined uronic acid residues (Part I, 141). Taking a value of $4.3\text{-}4.5 \times 10^{-2}$ mol. $HCOOH/C_5H_8O_4$ unit, obtained by extrapolating the curve to zero time, assuming the molecule to be unbranched and for the uronic acid residues present to release no "normal" formic acid due to the single methoxyl group carried, this indicates one xylose non reducing "end" group per 67-70 other residues (the latter being calculated as xylose residues). For a straight chain molecule containing unmethylated uronic acid residues, a value of one non reducing xylose "end" group per 89-93 other residues (calculated as xylose residues) is indicated.

In view of the overoxidation effects involved it is not intended to place much significance on the value of

67-70 for D.P. quoted.

Reducing Power.

An estimation of the chain length of the hemicellulose was obtained by reducing group assay in the same manner as was employed for the wheat straw xylan (Part I). The defects of the methods used, and the validity of the results obtained have already been discussed. A value corresponding to one reducing group per 130-144 residues (calculated as xylose residues) was obtained by oxidation with buffered alkaline hypoiodite, and a D.P. of 167-197 xylose residues by the colorimetric method of Meyer, Noelting and Bernfeld.

Methylation.

The hemicellulose A was methylated twelve times under nitrogen by the method of Haworth using dimethyl sulphate and sodium hydroxide (207). The partially methylated xylan obtained was slightly soluble in hot water. Inorganic salts were removed by dialysis, since solutions of the polysaccharide in chloroform showed a marked tendency to gel. The partially methylated material was not completely soluble in methyl iodide with the addition of small quantities of chloroform, methanol or acetone. It was therefore afforded a further five methylations with dimethyl sulphate and sodium hydroxide under nitrogen, and inorganic material was removed by washing, the product now being insoluble in water. The product was given three methylation by the Purdie method yielding 14.65 g. of methylated xylan; methoxyl content, 38.2%.

The methylated xylan was fractionated with various

mixtures of boiling chloroform:light petroleum, b.p.100-120°. As with the methylated straw xylan, the crude material was all soluble in a mixture of these solvents in the ratio 30/70 (v/v). The pure, fully methylated fraction representing 87% of the total, crude methylated polysaccharide had OMe, 38.6%; $[\alpha]_D^{19}$ -75° (c, 0.234 in chloroform); $[\alpha]_D^{20}$ -113° (c, 1.25 in m-cresol).

The viscosity of the pure, fully methylated xylan was measured in m-cresol at 20° ± 0.1° in an Ostwald viscometer. Using the K_m derived for methylated cellulose in this solvent, 12 x 10^{-4} (174), and assuming an "ideal" molecule, a value for M.W. of 8960 corresponding to a D.P. of 50 xylose residues was obtained. This value may be expected to be lower than the true D.P. since the molecule is heavily branched, i.e. carries a number of dependent uronic acid residues. Allowing for this fact, the value obtained above is in approximate agreement with that obtained for the same derivative by isothermal distillation ; (M.W., 11,100 ± 500; D.P. 66-72 residues).

Hydrolysis of Methylated Hemicellulose A.

The fully methylated hemicellulose was hydrolysed with methanolic hydrogen chloride, followed by dilute hydrochloric acid. The mixture of methylated sugars obtained was separated on a cellulose column into the following fractions, using as eluant a mixture of n-butanol: light petroleum b.p. 100-120° (35:65) saturated with water. Identification of the components is described later.

Table II.

1A	2:3-dimethyl methyl β D xyloside	0.022 g.
2A	2:3:4-trimethyl D xylose	0.046 g.
3A	Uronic acid	0.056 g.
4A	2:3-dimethyl D xylose Uronic acid	0.121 g.
5A	2:3-dimethyl D xylose Monomethyl D xylose Uronic acid	2.988 g.
6A	Uronic acid	0.321 g.

Uronic acid, the heavy concentration of which caused the column to "streak", accompanied all fractions after 3A. Recovery from the separation was 90-1%.

An attempt was made to separate fraction 4A into its constituents by exhaustive extraction, with chloroform, of the dimethyl sugar from the barium salt of the uronic acid. This was only partially successful, most of the uronic acid being obtained chromatographically pure, but a small quantity still remained with the dimethyl fraction. Fraction 5A and the mixed part of fraction 4A were combined and applied to a second cellulose column using as eluant butanol:ethanol:water (4:1:5) solvent containing ammonium hydroxide (1%) to retain the uronic acid at the head of the column. Separation was again incomplete, streaking being caused, in this case, by a drop in temperature during running which caused the eluting solvent to separate into two phases. The uronic acid was, however, obtained pure. Fractions were (1B) 2:3-dimethyl D xylose, 2.108 g., (2B) 2:3-dimethyl D xylose and 3-methyl D xylose, 0.390 g., (3B) Uronic

acid, 0.242 g., giving a total recovery from the second cellulose column separation of 91%.

The mixture of sugars constituting fraction 2B was re-separated on a third cellulose column, using as eluant n-butanol:light petroleum b.p. 100-120° (30:70) saturated with water. By this means, fractions (1C) 2:3-dimethyl D xylose, 0.112 g., and (2C) 3-methyl D xylose, 0.252 g. were obtained. Recovery from the third column was 93-4%. A complete separation of the components of the original hydrolysis mixture was thus finally obtained.

The methylated uronic acid from fraction 3B was shown to be present in part as the methyl ester. This was indicated by its high methoxyl content, lack of acidity and negative barium content, and by its behaviour paper chromatographically in various solvent systems. Fraction 3B was therefore hydrolysed and the methylated uronic acid recovered was found to be identical with that from the other fractions. All the uronic acid fractions were therefore combined, as were the various dimethyl fractions. Total yields of pure material obtained are given in Table III, together with a column giving the yields corrected for losses at the various stages. The methylated uronic acid methyl ester above was probably carried over from the reclaimation of hydrolysed material from the silver residues after neutralisation of the hydrolysis mixtures (see p. 104).

Sugar.	Pure material obtained.	Corrected weight.	Mol.% in hydrolysate.
2:3:4-trimethyl D xylose	46.0 mg.	50.9 mg.	1.34%
2:3-dimethyl D xylose	2.240 g.	2.7501 g.	78.16%
Monomethyl xylose	252 mg.	327 mg.	10.08%
Tetramethyl aldobiuronic acid	682 mg.	792 mg.	10.49%

Examination of the Fractions.

Fraction 1A.

This crystallised on removal of the solvent. The white needle crystals had m.p. 63, $[\alpha]_D^{16}$ -47.3° (c, 0.148 in water). This fraction was shown to be 2:3-dimethyl methyl β D xyloside by its hydrolysis to 2:3-dimethyl D xylose. This sugar has not previously been obtained crystalline, but a value for $[\alpha]_D^{20}$ of + 61.8° falling to + 43° (in methanol + 0.8% hydrochloric acid) is quoted for the equilibrium mixture of α and β 2:3-dimethyl methyl D xylosides (108). A mixture of the α and β forms of 2:3-dimethyl methyl D xylosides prepared from authentic 2:3-dimethyl xylose was seeded with the above crystals and stored at 0°. The syrup partly crystallised in the same form, but attempts to separate crystals from syrup by various means failed, since the m.p. of the crystals in the αβ mixture was below room temperature.

Fraction 2A.

This was shown to be 2:3:4-trimethyl D xylose. The fraction crystallised and the colourless crystals obtained had the correct m.p., mixed m.p., rotation, and methoxyl content for 2:3:4-trimethyl D xylose.

Hypoiodite oxidation indicated 99% pure aldopentose, and chromatographic examination gave a single discrete spot, R_G, 0.94. The derived anilide had the correct m.p., and mixed m.p. for 2:3:4-trimethyl D xylose anilide.

Fractions 1B and 1C.

These were identified as 2:3-dimethyl D xylose by rotation, methoxyl content, chromatographic examination, and by preparation of the crystalline anilide. The 2:3-dimethyl D xylose had a purity by hypoiodite oxidation of 97-8%, and crystallised slowly to a large, colourless mass of plates, m.p. 78°; mixed m.p. undepressed with an authentic sample (178). The derived anilide had the correct m.p. for 2:3-dimethyl D xylose anilide.

Fraction 2C.

This had a purity by alkaline hypoiodite of 97%, and the methoxyl content of a monomethyl pentose. It had $[\alpha]_D^{20}$ +17° (c, 0.8 in water). [3-methyl D xylose has $[\alpha]_D^{17}$ + 19° (c, 1.58 in water), (206) and 2-methyl D xylose, $[\alpha]_D^{20}$ + 34° (c, 0.9 in water), (139)]. The syrup gave what appeared to be a single spot, R_G, 0.38 on chromatographic examination on a long (66 hrs.) paper. On spraying the chromatogram with aniline oxalate solution, however, the colour obtained was intermediate between that of 2-methyl xylose and 3-methyl xylose standard samples which had been similarly treated. On spraying the chromatogram with a solution of p.anisidine hydrochloride in n-butanol, the fraction appeared identical in colour with a similarly treated 3-methyl xylose standard, (yellow-brown). Standard 2-methyl xylose gave a purple coloured spot with this

reagent. Ionophoretographic examination (400 v.; 4 hrs.) of the fraction on borate impregnated paper against standard 3-methyl xylose and 2-methyl xylose samples showed the fraction to be a mixture of these sugars in the approximate ratio of 20-25:1, estimated visually. There is a wide difference between both the M_G values and the colours obtained from these two sugars under the conditions used, and, by this means, traces of one sugar in a sample of the other may readily be detected. At pH 9.97, 3-methyl D xylose migrated almost twice as far as the 2-methyl isomer. (Distances moved at 400 v., after 3 hrs., 3-methyl D xylose: 10.1 cms., 2-methyl D xylose: 5.7 cms.). That migration should be markedly more rapid when the 2 position is free, allowing for complex formation to take place across C_1, C_2 in the ring form, is in agreement with previous findings for the derivatives of glucose and galactose (104, 209). Where the 2 position is blocked, as in 2-methyl xylose, and the ring form of the sugar has no other two available cis hydroxyls, interaction with boric acid may be able to take place through the aldehydo form under the alkaline conditions used for ionophoresis. This may be taken to account for the considerable migration of 2-methyl xylose observed. Similar findings have been noted for 2-methyl glucose (104). It may be noted that the interaction of the open chain form of the sugar with boric acid, in alkaline media, is not necessarily restricted to those cases in which cis hydroxyls are not available in the ring form (209).

The ratio of the two isomers indicated by ionophoresis

is in agreement with the data obtained chromatographically on fraction 2C, with its rotation, and with the fact that the fraction did not crystallise, even on seeding with 3-methyl xylose crystals and after long storage.

The anilide of fraction 2C was prepared, and, on seeding the syrup obtained with crystalline 2-methyl D xylose anilide, crystals having the correct m.p. for 3-methyl D xylose anilide were obtained.

Partly Methylated Uronic Acid.

The partly methylated uronic acid gave, on chromatographic examination, a cherry red, heart shaped spot travelling close to the starting line in n-butanol:ethanol: water (4:1:5). It had equivalent 399, and $[\alpha]_D^{19}$ + 51.5° (c, 0.78 in water), OMe, 32.3% (Calc. for $C_{15}H_{26}O_{11}$, equiv., 382; OMe, 32.5%; calc. for $C_{22}H_{37}O_{15}$, equiv., 541; OMe, 34.2%). The methoxyl content was thus correct for a tetramethyl aldobiuronic acid comprising a pentose and a hexuronic acid residue, although the equivalent was a little high.

Demethylation of a sample with hydrobromic acid (171) and examination of the product paper chromatographically indicated xylose to be the only simple sugar present. Traces of unmethylated and partly methylated uronic acids were also detected.

Reduction of the uronic acid glycoside ester with lithium aluminium hydride in ether, and hydrolysis of the product, gave a mixture of sugars which, on paper chromatographic examination against the relevant standards, appeared to be an approximately equal mixture of 2:3:4-trimethyl glucose and 3-methyl xylose. Traces of a disaccharide

due to incomplete hydrolysis of the reduced product, and possibly of 2:3-dimethyl xylose due to the presence in the unreduced fraction of a trace of methylated oligosaccharide-uronic acid, were also seen.

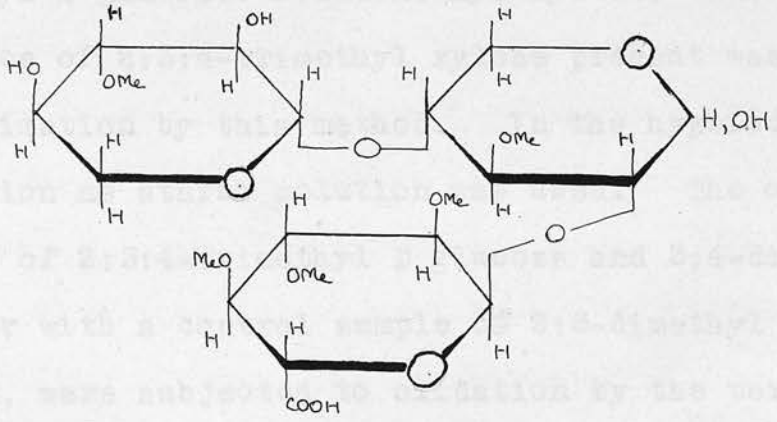
The mixture of 2:3:4-trimethyl glucose, and 3-methyl xylose was separated chromatographically on a thick filter sheet, and the sugars named were recovered as syrups in a molar ratio of 1.15:1.00. The 3-methyl D xylose syrup, which had the correct methoxyl content for a monomethyl pentose, crystallised on seeding with 3-methyl D xylose. The crystals obtained had the correct constants for 3-methyl D xylose, and their identity was confirmed by examination of the syrup against standard 2-methyl D xylose and 3-methyl D xylose chromatographically, and ionophoretically. Ionophoretic examination, under the conditions already described, showed the syrup to be entirely 3-methyl xylose, no 2-methyl xylose being detected on heavy sampling.

The 2:3:4-trimethyl D glucose, which had the methoxyl content of a trimethyl hexose, was characterised as the crystalline 2:3:4-trimethyl methyl β D glucoside.

Further methylation and reduction.

A sample of the tetramethyl aldobiuronic acid obtained from the column separations was fully methylated with methanolic hydrogen chloride, and with silver oxide and methyl iodide (x 2). The fully methylated uronoside methyl ester was reduced with lithium aluminium hydride in ether and the product was hydrolysed. Chromatographic examination of the mixture produced, using a spray of p.anisidine in n-butanol showed 2:3:4-trimethyl glucose and 3:4-dimethyl xylose to

be present in approximately equal amounts, together with a little 3-methyl xylose and a trace of 2:3:4-trimethyl xylose. No 2:3-dimethyl xylose was present. The 3-methyl xylose present was due to undermethylation, or demethylation on hydrolysis, whilst the 2:3:4-trimethyl xylose had origin in the trace of oligosaccharide-uronic acid previously shown to be present in the uronic acid fraction. The distinct trace of 2:3:4-trimethyl xylose present, in comparison to the negligible amount of 2:3-dimethyl xylose detected from reduction of the partly methylated uronic acid from the column separation, makes it possible that some of the uronic acid fraction may have been present in the hydrolysis mixture in the form I.



I.

This would account for the above fact, and, in addition

(a) the slightly high equivalent obtained for the partly methylated uronic acid, but absence of 2:3-dimethyl xylose on hydrolysis of this fraction, or on reduction and hydrolysis of the fraction.

(b) the slightly high proportion of 3-methyl xylose obtained on reduction of fraction 4, and separation of the component sugars after hydrolysis (3-methyl D xylose; 2:3:4-trimethyl D glucose, 1.15:1.00).

Attempts were made to detect formaldehyde liberated from the partly methylated uronic acid using periodate, glycol, and Schiff's reagent on paper chromatograms (172). Such a procedure had been successfully followed with the partly methylated uronic acid from methylated wheat straw xylan (Part I). Unambiguous results could not, however, be obtained.

The mixture of sugars produced above by the methylation, reduction and hydrolysis of the partly methylated uronic acid fraction was separated on a long, thick filter sheet, and the components were estimated with alkaline hypoiodite. Molar ratios of 1.0:0.95:0.48 were obtained for 2:3:4-trimethyl D glucose: 3:4-dimethyl xylose; 3-methyl D xylose. The trace of 2:3:4-trimethyl xylose present was too small for estimation by this method. In the hypoiodite estimation no starch solution was used. The oxidised samples of 2:3:4-trimethyl D glucose and 3:4-dimethyl xylose, together with a control sample of 2:3-dimethyl D xylose and a blank, were subjected to oxidation by the periodate ion for 45 hours. After this time, a colorimetric test for formaldehyde was performed on all samples using potassium ferricyanide and phenylhydrazine hydrochloride (176). Formaldehyde was shown to be present in the oxidised 2:3-dimethyl D xylose control, and in the oxidised 2:3:4-trimethyl D glucose sample. The absence of formaldehyde in the oxidised 3:4-dimethyl xylose sample was taken as added confirmation of the fact that this dimethyl xylose was, in fact, the 3:4- isomer.

The proportion of 3-methyl D xylose present as

estimated by hypoiodite is greatly in excess of that to be expected from chromatographic examination of the mixed syrup. This may have been due to the small excess of iodine used being insufficient to oxidise stoichiometrically the amounts of 2:3:4-trimethyl D glucose and 3:4-dimethyl xylose present. If these two sugars were present in approximately equal molar ratios, the percentages oxidised under the conditions used would be comparable, although not 100%. Thus results in agreement with a 1:1 molar ratio for these two sugars might be obtained, with an apparently high value for the smaller percentage of 3-methyl D xylose present. That the trimethyl glucose and 3:4-dimethyl xylose should be present in 1:1 molar ratio on full methylation, reduction and hydrolysis of the partly methylated uronic acid fraction, necessarily follows from the results of reduction and hydrolysis of the partly methylated uronic acid fraction, and separation of the component sugars. The analyses of the partly methylated uronic acid fraction, and the extremely low quantity of trimethyl xylose isolated after methylation, reduction, and hydrolysis of the partly methylated uronic acid fraction lead to the conclusion that the latter fraction was almost completely aldobiuronic acid with a trace , only, of oligosaccharide-uronic acids units. (The trace of oligosaccharide-uronic acid present may be assumed to be in the form I p. 178).

The methylation, reduction and hydrolysis of the partly methylated uronic acid fraction provides results to fix the linking between xylose and glucuronic acid residues, in the aldobiuronic acid unit, from position 1 in the glucuronic

acid to position 2 in the xylose residues. This follows since the only dimethyl xylose obtained, after the above procedure, was the 3:4- isomer.

Examination of Acidic Fraction of Hemicellulose A.

Hemicellulose A was hydrolysed under controlled conditions, and the barium salt of the aldobiuronic acid was partially separated from xylose in the neutral hydrolysate by precipitation of the salt in ethanol. Complete separation of the uronic acid from the xylose, in a chromatographically pure condition, by precipitation of the former as the barium salt was not found to be practicable. The final mixture of barium uronate and xylose obtained contained approximately 50/50, xylose/barium uronate when examined paper chromatographically (visually). It had equivalent 384; OMe, 4.95%; the corresponding constants for the barium salt of a monomethyl aldobiuronic acid being 340, and 7.6%. The molar ratio methoxyl/uronic anhydride found is thus 0.74/1.0. The molar ratio methoxyl/uronic anhydride for the unhydrolysed hemicellulose A is 1.0/1.0.

The conclusion that one quarter of the methyl uronic acid residues were demethylated during isolation of the barium aldobiuronate is unjustified, since on reduction and hydrolysis of the barium salt, the ratio of glucose to 4-methyl glucose obtained was much lower than 1:4. It may be mentioned that similar figures have been quoted by Bishop (202) for the demethylation of barium monomethyl aldobiuronate on isolation.

Hydrolysis of crude barium aldobiuronate.

The crude barium aldobiuronate was subjected to vigorous methanolysis in a sealed tube for 20 hours at

70-80° with 16% methanolic hydrogen chloride. The product was in part hydrolysed with 0.5N hydrochloric acid at 100° for 4 hours. The neutral syrup was examined paper chromatographically in two acid solvent systems. Some 4-methyl glucuronic acid, glucuronic acid, and xylose were shown to be present.

Reduction of crude uronoside methyl ester with lithium aluminium hydride.

The remainder of the syrup from the methanolysis treatment above was reduced with lithium aluminium hydride in ether. After hydrolysis of the product with sulphuric acid (1N; 3 hrs.) at 100°, and examination paper chromatographically in various solvents, xylose (in quantity) was observed, together with traces of glucose and of glucurone. No 4-methyl glucose could be detected. Some unreduced uronic acid was also observed. Since 4-methyl glucuronic acid had been shown to be present in the mixture before reduction, the absence of 4-methyl glucose from the reduction products was possibly due to the comparative insolubility of 4-methyl glucuronic acid, compared with glucurone, in the dry ether.

Reduction of pure aldobiuronoside methyl ester with lithium aluminium hydride.

A sample of crude barium aldobiuronate was purified by separation on a thick filter sheet. The pure barium salt was converted into the uronoside methyl ester, which was reduced with lithium aluminium hydride in dry, boiling dioxan. The reduced product, after gentle hydrolysis and neutralisation, was examined paper chromatographically in

various solvents. Xylose, and 4-methyl glucose were identified, but no glucose could be detected. Dioxan was found to be a better solvent for the above reduction than ether. Even so, most of the aldobiuronic acid originally present appeared unchanged in the final product.

Hydrolysis of pure barium aldobiuronate.

A sample of pure, dry barium aldobiuronate, prepared by separation of the crude barium aldobiuronate on thick filter sheet, was methanolysed under fairly vigorous conditions (5% MeOH/HCl; 60°; 24 hrs.) and hydrolysed with hydrochloric acid (1.8N; 100°; 18 hrs.). On neutralisation, and examination of the mixture paper chromatographically in various solvents, xylose, glucurone, glucuronic acid, and 4-methyl glucuronic acid were identified. Xylose was heavily predominant, being present in much heavier concentration than the combined total of all other sugars, whilst the combined amounts of glucurone and glucuronic acid present were in excess of the 4-methyl glucuronic acid.

This result, and the result of the hydrolysis of the crude barium aldobiuronate, would appear to show that although extensive demethylation does take place, some methyl glucuronic acid may be detected in the hydrolysis products, even after hydrolysis under the most vigorous conditions. That extensive demethylation of a methyl hexuronic acid residue is effected under the conditions necessary to hydrolyse the aldobiuronic acid linkage has previously been reported (132).

Reduction of pure aldobiuronoside methyl ester with sodium borohydride.

Pure barium aldobiuronate was converted to the uronoside methyl ester and reduced with sodium borohydride in water at room temperature. Hydrolysis of the resultant syrup, and examination of the product paper chromatographically, showed 4-methyl glucose and xylose to be present, in good yield and approximately equal quantities. A trace of glucose, just observable on the paper chromatogram was seen. No other sugars or uronic acids were seen. The mixture of xylose and 4-methyl glucose was separated on filter sheet and the osazone of the monomethyl derivative was prepared. This had the correct m.p. and mixed m.p. for 4-methyl D glucosazone. An X-ray powder photograph of the osazone (by kind permission of Dr. C. Beevers) gave a result identical with that obtained from a sample of 4-methyl D glucosazone prepared from synthetic 4-methyl D fructose (215).

The uronic acid residue present in hemicellulose A was therefore 4-methyl glucuronic acid.

Isothermal distillation of Methylated Hemicellulose A.

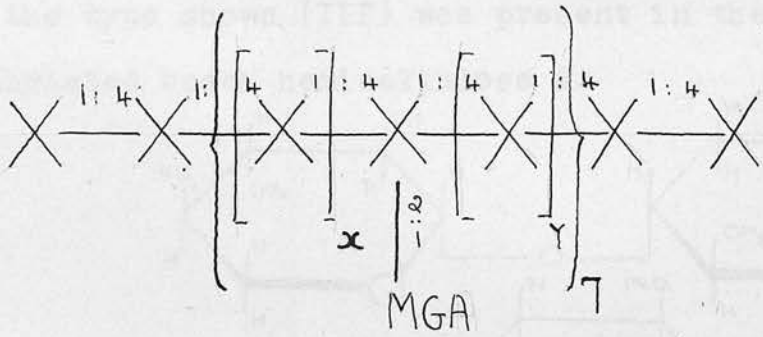
The M.W. of methylated hemicellulose A (fraction 5) was determined by isothermal distillation of a solution in benzene (0.028 g. in 4.025 g. benzene) at 22.5°, by courtesy of Mrs. H. Zienkiewicz B.A. A value of 11,100 ±500 was obtained, corresponding to a D.P. of 66-72 xylose residues.

Structure.

On the basis of the results described, a probable

structure for hemicellulose A is suggested below:-

A straight chain of approximately 67 D xylopyranose residues linked β 1:4 with seven 4-methyl D glucuronic acid residues attached to main chain xylose residues through the 2 position in the latter, (see II).



$x+Y$ = 8 approx.

X = D xylopyranose.

MGA = 4-methyl D glucuronic acid.

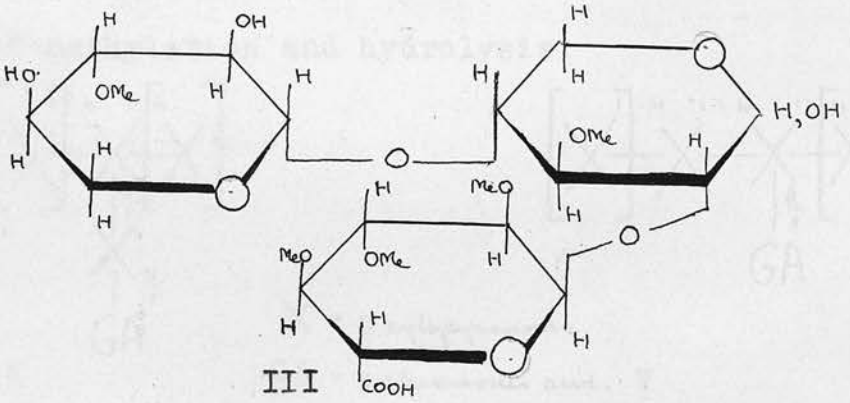
II

As in the case of wheat straw xylan, the quantity of monomethyl xylose isolated from the hydrolysis products of the methylated material closely approximates the amount of aldobiuronic acid present (monomethyl xylose, 10.08%; tetramethyl aldobiuronic acid, 10.49%, molar). The suggestion is made that the large, partly methylated aldobiuronic acid methyl ester grouping may cause hindrance to full methylation of the adjacent main chain xylose residue. Factors which appear to operate against the explanation that the monomethyl xylose is due to casual undermethylation, or demethylation on hydrolysis, are,

(a) Where the uronic acid residue is linked to position 3 in main chain xylose residue (i.e. in wheat straw xylan), the monomethyl xylose isolated is predominantly 2-methyl

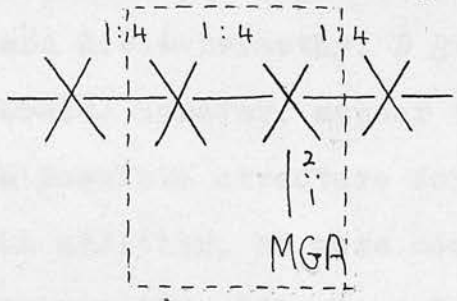
D xylose; and where the uronic acid is linked to position 2 in the main chain xylose residue (i.e. beechwood xylan), the monomethyl xylose isolated is almost exclusively the 3-methyl isomer.

(b) Some evidence exists (see p. 178) that a triose unit of the type shown (III) was present in the hydrolysis of methylated beech hemicellulose A.

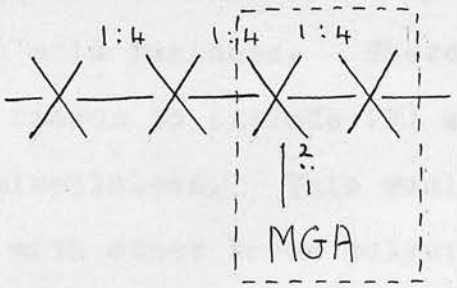


III

From results already quoted, it is clear that this unit must have had origin in A and not in B.



A.

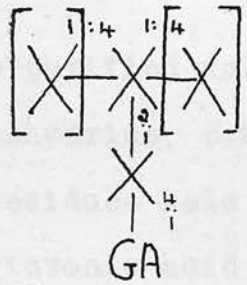


B.

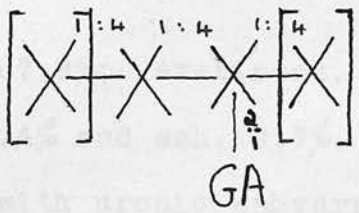
In fact, no triose unit such as B was traced in the hydrolysis products of the methylated hemicellulose. (The existence of B would have been at once revealed by the appearance of 2:3-dimethyl xylose in the products of methylation, reduction, and hydrolysis of the unit).

It may be noted that although the usual position of the branching point in polyuronide xylans, and xylans so far examined has been from position 3 in the main chain xylose residue (140, 141, 202, Pt.I.), the existence of branched

polyuronide xylans where branching is from the 2 position have been reported. Thus Phormium tenax hemicellulose (143) yields a polyuronide xylan in which the uronic acid is joined to the main chain through the 2 position in a xylose residue in the latter. McIlroy has postulated a structure (IV) for the hemicellulose since an aldotriuronic acid resistant to methanolysis was isolated from the products of methylation and hydrolysis.



IV



X = D xylopyranose.
GA = D glucuronic acid. V

The methylated aldotriuronic acid unit isolated was shown to contain 2:3-dimethyl D xylose; 3-methyl D xylose, and 2:3:4-trimethyl D glucuronic acid residues. There would, however, appear to be no reason to exclude (V) as a possible structure for the hemicellulose. This would, in addition, be more comparable with other known polyuronide xylans (98, 141, Part I).

An aldobiuronic acid isolated from aspen wood (208) has also been shown to be a glucuronic acid residue linked to xylose at position 2 in the latter. The glucuronic acid residue, in this case, carried a single methoxyl grouping at position 4, and was thus identical with the aldobiuronic acid isolated from the beechwood hemicellulose A in the present investigation i.e., 2-(4-methyl D-glucuronosyl)-α-D-xylose. In view of this, a closer examination of the polyuronide xylan present in aspen wood which gives rise to this aldobiuronic acid might prove to be of interest.

SUMMARY.

I. Wheat Straw Hemicellulose.

1. The hemicellulose (xylan) was extracted from defatted, delignified wheat straw with dilute alkali. It contained xylose, and arabinose (6.2%) together with some uronic acid.

2. Complete removal of arabinose by purification through the copper complex was found to be impossible. Purification was finally effected by fractional solubility in aqueous ethanol.

3. The purified xylan had xylose, 87.9%; arabinose, 0.6%; uronic anhydride, 3.2%; methoxyl, 0.4% and ash, 0.7%. The xylose residues held in combination with uronic anhydride as aldobiuronic acid are not taken into account in these figures.

4. The reducing power determined by alkaline hypoiodite indicated a D.P. of 164, and by 3:5-dinitro salicylic acid, 43. Neither method is completely reliable.

5. The fully methylated xylan had a viscosity in m.cresol corresponding to a D.P. of 26-7.

6. The methylated xylan on hydrolysis and estimation with alkaline hypoiodite yielded the following molar ratios; trimethyl pentose, 2.8%; 2:3-dimethyl xylose, 94.3%; monomethyl xylose, 2.9%. The uronic acid was not estimated.

7. The hydrolysate of the methylated xylan was separated on a cellulose column. The following were identified, calculated as 100% total pentose; 2:3:4-trimethyl xylose, 2.4%; 2:3-dimethyl xylose, 94.5%; 2-methyl xylose, 3.4%.

8. In addition to the above, there was obtained a uronic acid identified as 2-methyl-3-(2:3:4-trimethylαD glucuronosido) D xylose.

9. Unmethylated xylan was hydrolysed, the aldobiuronic acid separated as the barium salt, and rehydrolysed. Glucuronic acid and glucurone were detected, but no 4-methyl glucuronic acid was found.

10. On the basis of the above results, and the results of periodate oxidation, a probable structure is suggested for the hemicellulose.

II. Beechwood Hemicellulose A.

1. The hemicellulose (xylan) was extracted with dilute alkali from pre-extracted beechwood.

2. The xylan gave on hydrolysis, xylose, 81.6%; rhamnose, 0.40%; and had uronic anhydride 9.5%; methoxyl, 1.7%; and total lignin, 1.9%.

3. The reducing power, by alkaline hypoiodite, indicated a D.P. of 130-144, and by Meyer's method, 167-197.

4. The viscosity of the fully methylated xylan in m.cresol indicated a D.P. of 50.

5. The hydrolysate of the methylated xylan was separated on a cellulose column. The following molar ratios were obtained:- 2:3:4-trimethyl xylose, 1.34%; 2:3-dimethyl xylose, 78.16%; monomethyl xylose, 10.08%. No rhamnose derivatives were obtained. The monomethyl xylose contained 3-methyl xylose and 2-methyl xylose in ratio 20:1 approx.

6. In addition to the above, 10.49% (mol.) of a methylated aldobiuronic acid was obtained from the column and identified as 3-methyl-2-(2:3:4-trimethylαD glucuronosido) D xylose.

7. The unmethylated xylan was hydrolysed and a monomethyl aldobiuronic acid was isolated as the barium salt. Reduction and hydrolysis of the acid yielded 4-methyl D glucose,

8. Periodate oxidation of the unmethylated polysaccharide gave an uptake of 1.0 mol. periodate per $C_5H_8O_4$ unit. The formic acid released did not reach a constant value.

9. On the basis of the above results, a probable structure is suggested for the hemicellulose.

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